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# THE SMALLEST LIVING THINGS

*Life as Revealed by the Microscope*



By GARY N. CALKINS, Ph. D., Sc. D.

PROFESSOR OF PROTOZOLOGY, COLUMBIA UNIVERSITY

and at the

MARINE BIOLOGICAL LABORATORY



*Highlights of Modern Knowledge*

PROTISTOLOGY



THE UNIVERSITY SOCIETY  
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To

*My three sons*

HENRY SEYMOUR,  
GARY NATHAN,  
AND  
SAMUEL WILLISTON

*in affectionate remembrance  
of happy days together*

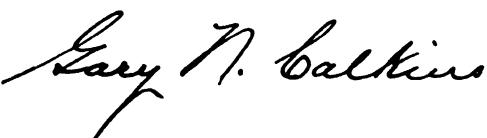


## PREFACE

**I**N PREPARING the manuscript for this book of the Series I have tried to avoid, as far as possible, all strictly technical matters which do not carry their own explanations. I have taken advantage of this opportunity, however, to put into print for the first time a point of view which may come as a shock to most protozoologists and may cause many protests. I refer to the transfer of all chlorophyll-bearing flagellates from the classification of Protozoa to the botanical classification of Algae. This procedure is due to no sudden whim on my part to give novelty and originality to the book, but is the result of careful consideration and full conviction and has been taught for several years in my class work.

The little book is a brief survey of a vast field of living things which are unnoticed by and are practically unknown to the average person. It is not a catalogue of minute forms of animal and plant life, nor is it a guide to the fascinating mysteries revealed by the microscope; rather it is written as a basis for reflection on some of the fundamental problems concerned with the mechanisms and activities of living substance. It takes for granted the exclamations and superficial delights of the first few hours with a microscope and with its revelations of kaleidoscopic movements, colors, and forms, and invites the reader to look beyond these to the meaning of it all. I submit again what was written in the introduction of Rösel von Rosenhof's *Insekten Belüstigungen* (1744) :

Lies dieses Buch, und lern dabey,  
Wie gros Gott auch im Kleinem sey.\*

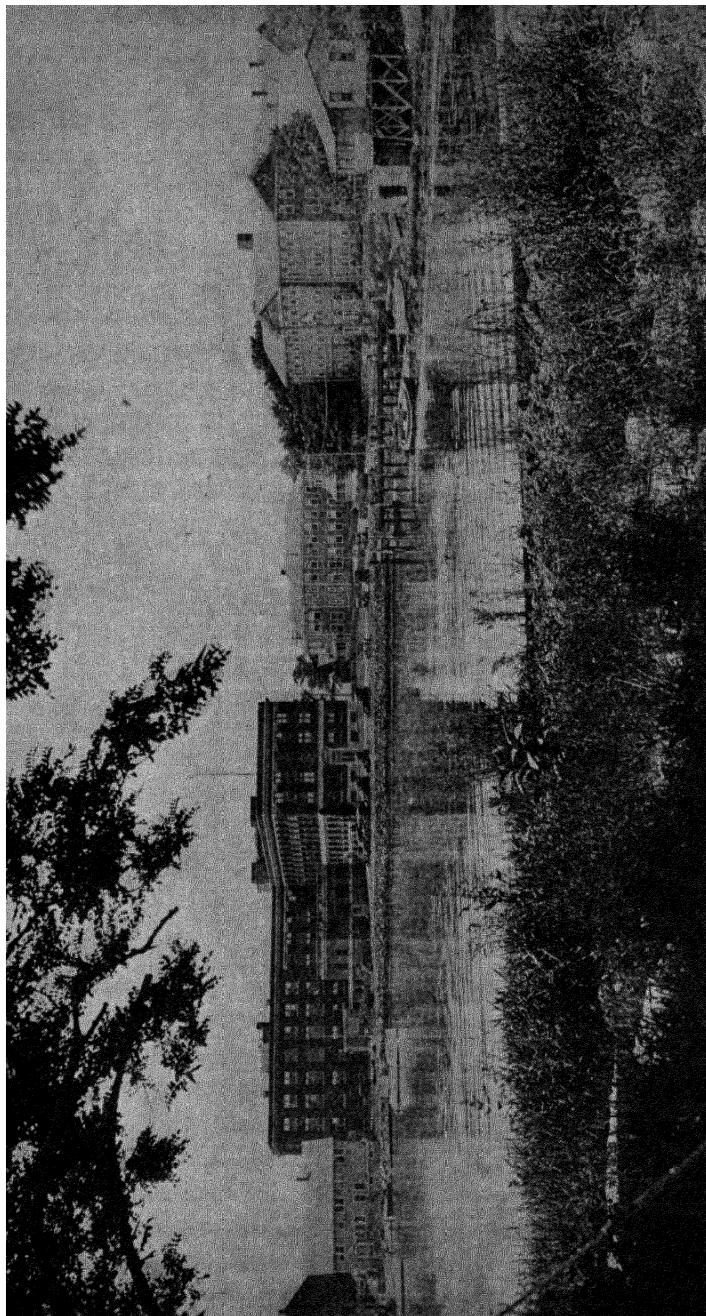


Columbia University,  
October, 1932.

\* This beautiful thought, freely translated is:

Read this book, and learn therefrom  
How God is also great in little things.

—Editor



MARINE BIOLOGICAL LABORATORY

Woods Hole, Massachusetts

*From a photograph by Professor F. E. Chidester*

## CHAPTER I

### OUR APPROACH

#### AN OPEN AND UNBIASED MIND IS NECESSARY

**I**N APPROACHING the realm of biology as a science it is well to discard all pre-conceived ideas of life, traditions as to its origin, and all metaphysical speculations. Viewing things objectively is a safeguard against shock to prejudice or to religious belief. We must realize that man cannot get outside of himself in the interpretation of the universe and that he is dependent upon his senses and reasoning power in the formulation of concepts of matter, space, and time. We must further realize that these concepts change as newer evidence gained by the senses and by reasoning increases, and we must therefore conclude that, at present, final causes are unknowable, that so-called "laws" are not final, and that all knowledge is relative.

To enter the realm of biology in this way is to bring an open and an unbiased mind to the contemplation of living things. In biology, as in other branches of exact knowledge, we find concepts which have the value of so-called facts, and concepts having the value of theories, or attempted explanations of the facts and their relationships. In the last analysis, and with the changing conceptions of atoms, matter, and energy which are resulting from increased knowledge, even our so-called facts may have to be relegated to the realm of theory, or they may be found to be based on theory, and as such to be merely relative. Yet such facts are the most stable concepts we have, and when based upon accurate observations afford a safe criterion of truth and a stimulus to further progress.

Facts, in this limited sense, are the building-blocks for further theories, and such theories become more or less permanent creations according to the number, variety, and universality of the facts which enter into their construction. Such, for example, is the conception established today and known as the doctrine of evolution.

## THE GROWTH OF KNOWLEDGE

Biology, briefly defined, is the science of life, or the knowledge of living things. Man has always been interested in the subject as offering some possibility of an interpretation of himself, his mechanisms, and his origin. In pursuit of this knowledge he has searched the remote parts of the earth; he has dug deep into its solid substance and has explored the depths of the ocean. He has studied and dissected every new type of living thing that has been found; he has compared its structures and its functions with those of organisms which were already known; he has given each such new type two names by which it may be known and identified; and he has arranged it, with other similar organisms, into an orderly system of classification.

## THE THOROUGHNESS OF BIOLOGICAL RESEARCH

It is perfectly safe to state that no one knows exactly how many animals and plants have been thus studied and classified; various estimates, running into millions, have been made. Not only the obvious structures which form the basis of the study of anatomy have been studied and compared, but the finest details of structures entering into the make-up of living substance have been analyzed with equal care, and the sciences of histology and cytology\* have been the result.

Not only structures but the activities or functions of living organisms have been followed out with equal painstaking thoroughness. Here again the more obvious functions were the first to be known and it was soon demonstrated that all animals and plants respire, requiring oxygen for the purpose, and giving off carbon dioxide as a result, that oxidation is the underlying phenomenon of all animal activities, and that waste matters result which must be disposed of. It has long been known that such activities involve the breakdown of living substance and that the loss is made good by the addition of food substances. The two series of activities—waste and repair—are comprised in the biological term *metabolism*, destructive metabolism comprising

---

\* Histology and cytology are two branches of biology, both of which deal with cells; histology treats of cells chiefly as the elements constituting tissues, while cytology treats of the individual cell with reference to its structure, function, multiplication, and life history.

all of the activities which have to do with the breakdown of substance, and constructive metabolism including all the activities which have to do with the repair of waste and with growth.

One of the remarkable consequences of the accumulation of all of these facts regarding structures and functions of living things, is the realization that *all animals are fundamentally alike rather than fundamentally different*. If we could analyze any one of the fundamental activities of living matter clear through to the end in any one organism, it would be a simple step to apply such knowledge to other organisms. Details would vary but the functions are essentially the same. So it is with the fundamental principles underlying modern study in genetics — principles formulated from the study of heredity in the fruit fly (*Drosophila*)\* are applicable to all types. In a similar way a great deal of light may be thrown on the nature of the functions of higher types of animals by the thorough study of the functions and activities of the Smallest Living Things.

Back in the 17th century, in the days of awakening imagination, when men were beginning to ask what is the meaning of life and how does it arise, the English physician, William Harvey, the Italian physiologist, Francesco Redi, and other enlightened naturalists of that age were experimenting independently on different types of animals and were rapidly putting an end to the current belief that flies and other common animals arise from dirt or lifeless matter by so-called spontaneous generation. As a result of such experiments it was demonstrated that flies lay eggs which develop into maggots, and maggots turn into flies; in other words that life comes from pre-existing life—a fundamental conception expressed by the aphorisms *omne vivum ex vivo* and *ex ova omnia*.

### THE FIRST APPEARANCE OF LIVING THINGS

Scientists have concluded that living things must have appeared on the earth more than 1200 million years ago.† Evidences of past life are afforded by the remains (usually structures such as shells, skeletons, woody fiber, etc.) of animals and plants embedded in some protective material which, subsequently, be-

\* See "Heredity and Variation," pages 51-66, in this Series.

† See "The Earth" and "Fossils" both in this Series.

came hardened into rock. Such remains are known as fossils.\* The oldest fossils are found in the lowest strata of rocks forming the earth's crust. Higher strata, deposited later, are found to contain types of organisms which lived at a later time. It is

*Courtesy of the American Museum of Natural History*

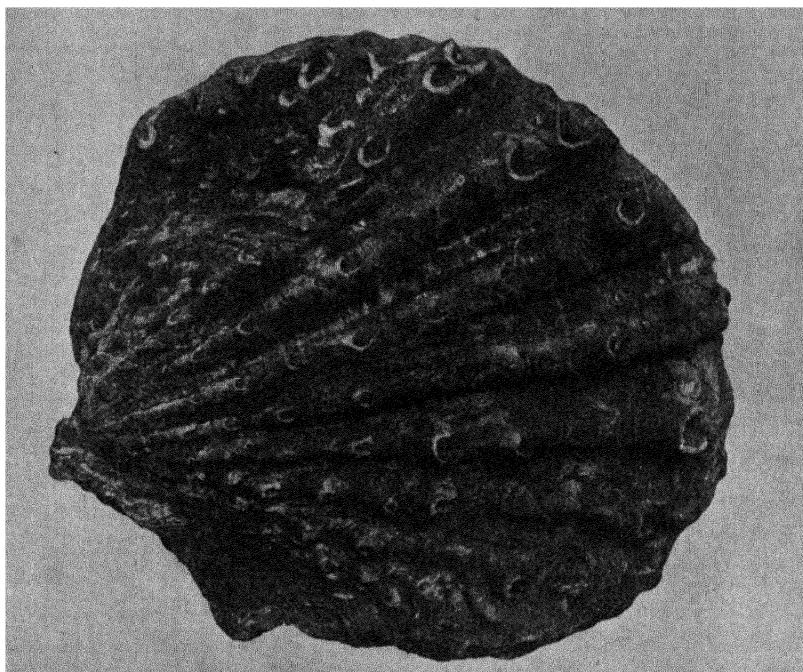


Fig. 1—A BRACHIOPOD  
*Spirifer (Dethysia) perlamellosus*, Hall

thus possible to obtain something of a record, though often rather indefinite and fragmentary, of the succession of types of living things from the earliest formed rocks to the present day.

Geologically, the history of the earth is divided into seven eras; and some of these are further subdivided into periods as shown in the accompanying table of Geologic Chronology. Paleontology is a branch of the biological sciences dealing mainly with fossil forms. Studies of these remains of past life led to the interesting conclusion that there is an increasing complexity

\* See "The Earth" and "Fossils" both in this *Series*.

## GEOLOGIC CHRONOLOGY

<i>Eras</i>	<i>Periods</i>	<i>Duration in millions of years</i>	<i>Order of appearance of animal forms</i>
CENOZOIC	Quaternary	1	First men together with list below.
	Tertiary	59	First monkeys and anthropoid apes
MESOZOIC	Cretaceous	40	First placentals; monotremes; marsupials
	Comanchian	25	
PALEOZOIC	Jurassic	35	First modern bony fish (teleosts); first birds, mammals
	Triassic	35	Rise of dinosaurs
PALEOZOIC	Permian	25	Great development of amphibia and reptiles
	Carboniferous	35	First air-breathing vertebrates
		50	First reptiles
PALEOZOIC	Devonian	50	First ganoids, lung fishes, etc.
	Silurian	40	Sharks and rays (cartilaginous fishes) First air-breathing invertebrates (scorpions)
PALEOZOIC	Ordovician	85	First evidence of vertebrates (early sharks, ostracoderms)
	Cambrian	70	Nearly all types of invertebrates; no vertebrates
PROTEROZOIC		650	First protozoa; calcareous algae; sponges; worms
		800	Indirect evidences of life
ARCHEOZOIC		600	
AZOIC		400	No evidence of life
COSMIC			

*Oldest era at bottom*

of living things with increasing thickness of the deposited portions of the earth's crust. Relatively simple forms came first and traces of their existence are to be found in the very earliest rocks (pre-Cambrian). In a general way this succession of forms is shown in the table.

### PRESERVATIONS IN ROCKS

It must not be forgotten that soft-bodied animals had but little chance of preservation in the rocks. Exceptional cases are fossils of certain types of jellyfish which are found in Cambrian deposits. Animals with hard parts, however, afford a fairly complete history. Protozoa with limestone shells (Foraminifera) or silicious skeletons (Radiolaria) which abound in our modern seas are well represented in the Lower Paleozoic and Proterozoic eras. Similarly, the much more complex Brachiopods (lamp-shells), some types of which are living today, flourished way back in the Cambrian period. One of them, Lingula, is apparently the same today as it was in the Silurian period millions of years ago. Representatives of our modern groups of sponges, corals, crustacea, mollusca, and spiders likewise first appeared at that time. Animals with a cartilaginous skeleton (sharks) first appeared in the Ordovician and Silurian periods; amphibia in the Pennsylvanian; reptiles in the Mississippian period; birds and first mammals in the Jurassic period; monkeys and anthropoid (man-like) apes in the Tertiary period; and the first men in the Quaternary period.

Fossil plants, representative of the ferns, similarly, are found as far back as the Devonian period, and flourished as great tree-like forms in the Carboniferous period. Flowering plants appeared in the Cretaceous period.

### WHERE AND HOW LIVING MATTER ORIGINATED

This rapid survey is adequate to show that living things must have appeared very early in the physical history of the earth. Where and how living matter originated, while not entirely idle speculation, are questions which have sometimes led to theories which have not been confirmed by experiment.

The creation of living things out of lifeless matter (spontaneous generation) has never been accomplished experimentally,

but it is probable that physical conditions in the early history of the earth were far different from conditions today and that life may have originated from inorganic matter in the past. This concept has never been expressed better than by T. H. Huxley in his essay on "Spontaneous Generation":

"And, looking back through the tremendous vista of the past, I find no record of the commencement of life, and therefore I am devoid of any means of forming a definite conclusion as to the conditions of its appearance. Belief, in the scientific sense of the word, is a serious matter, and needs strong foundations. To say, therefore, in the admitted absence of evidence, that I have any belief as to the mode in which the existing forms of life have originated, would be using words in a wrong sense. But expectation is permissible where belief is not; and, if it were given me to look beyond the abyss of geologically recorded time to the still more remote period when the earth was passing through physical and chemical conditions which it can no more see again than a man can recall his infancy, I should expect to be a witness of the evolution of living protoplasm from not living matter. I should expect to see it appear under forms of great simplicity, endowed, like existing fungi, with the power of determining the formation of new protoplasm from such matters as ammonium carbonates, oxalates, and tartrates, alkaline and earthy phosphates, and water, without the aid of light. That is the expectation to which analogical reason leads me; but I beg you once more to recollect that I have no right to call my opinion anything but an act of philosophical faith." \*

### PROTOPLASM IS LIFE

Future research may demonstrate the possibility of originating living protoplasm from matter that is not living, but there is no evidence whatsoever that it does so arise under conditions existing today. Step by step during the last three hundred years the aphorism *omne vivum ex vivo* (all life comes from life), which originated in the seventeenth century, has been extended to all types of organisms. The last step, taken in 1855 to 1855 by Pasteur, showed that the smallest known organisms, the

---

\* T. H. Huxley, *Lay Sermons, Addresses, and Reviews*. Edition 1883, page 366, D. Appleton and Company.

bacteria, like the largest, come from pre-existing organisms similar to themselves. If, as is generally believed, the concept expressed by the above aphorism, and upon which the entire science of heredity is based, is universally true, then it follows that all living things today represent protoplasm that has been continuously living since life commenced, and that the protoplasm of a minute ameba, visible only with the microscope, is just as old as the protoplasm of man.

### LOWER AND HIGHER ORGANISMS

If protoplasm was originally formed under special physical and chemical conditions, as conceived by Huxley, then it is possible that the protoplasms thus formed were not equally potent. Some types of that primordial protoplasm may have possessed in high degree what Osborn calls the "potential of evolution"—the capacity to develop into higher forms—while other types may have had this potential in lesser degree. In the long history of the earth the latter (*e.g.*, Protozoa which have persisted throughout) never advanced to high grades of specialization, whereas the former, because of their greater capacity for change, evolved into the complicated forms of animal and plant life found today. There is no reason to believe that "higher" animals have evolved from the forms that exist today as "lower" animals, but there is a possibility that all animals, "higher" or "lower" in organization, may have had a common but unequally potent type of ancestral protoplasm back in the protoplasm-forming age.

There is some logical basis for the popular conception of higher and lower types of organisms. When so used, however, these terms connote greater or less specialization in organ formation. Functionally, the same fundamental protoplasmic activities are present in all types, and the make-up of protoplasm, whether in high or low types, is, so far as we can determine, everywhere essentially the same. Chemically, protoplasm is a mixture of proteins, carbohydrates, fats, salts of different kinds, and water. The chemical and physiological activities of these substances between and among themselves and between them and the environment constitute the dynamic manifestations of life, or vitality. These are usually grouped into fundamental categories of activity under the headings *irritability, respiration, excretion,*

*nutrition*, and *reproduction* (See Chapters VIII and IX), all living things manifesting these activities in one way or another.

In the so-called metazoa (many-celled animals) certain parts of the body, the organs (such as heart, stomach, kidneys, etc.) are specialized to perform certain activities, and the "higher" the type of organism the greater is the specialization. Conversely, organisms of "lower" types have fewer organs and each organ does proportionately more varied physiological work.

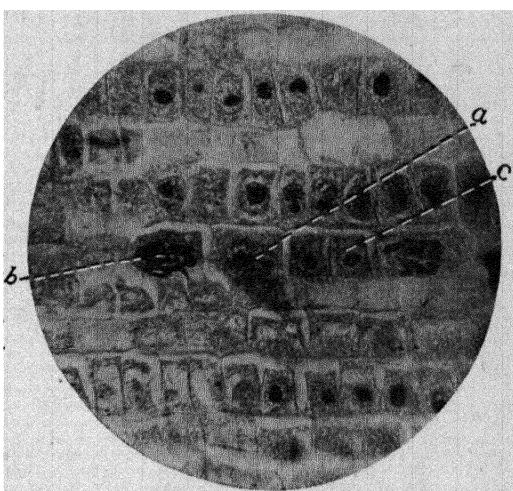
### ALL LIVING THINGS COMPOSED OF CELLS

In 1838 to 1839 a great step in the advancement of biological knowledge was taken by Theodor Schwann, a German anatomist,

Fig. 2—CELLS FORMING A TISSUE

Each cell has its own walls, nucleus, and cytoplasm or cell body which are formed by division of pre-existing cells. *a*, a cell with its nucleus in an early stage of division; *b*, a nucleus in the midphase of division; *c*, two young cells just after division.

From a photomicrograph by the author  
Magnification, 600



who, with the co-operation of Matthias Jakob Schleiden, a botanist, formulated the *Cell Theory of Organic Structure*.\* This is now a basic fact in biological science. All living things are made up of units of structure called *cells* (Fig. 2). Thus, a higher type of organism is not merely a larger mass of living protoplasm, but it is also composed of numerous minute units, or cells. The cells contain living protoplasm, through the activity of which the supporting structures found in all higher animals—cartilage, bone, shells, skeletons, wood, etc.—are formed. The cells are specialized to perform various functions and activities and are arranged in sheets, or groups, of like kinds, so that we

\* See "The Plant World" in this Series, pages 4-6.

have tissues, such as epithelial or covering tissue, muscle tissue, secreting or glandular tissue, germinal tissues, and the like. The special parts of an organism which we know as organs, are made up of tissues, and the tissues of cells. The functional activity of the organ as a whole is the sum total of the activities of its constituent cells. Thus, a higher organism is the aggregate of

specialized cells, each contributing its part to the harmonious working of the whole.

In "lower" metazoa this division of physiological labor is less complete; each of the constituent cells has more work to do to maintain the fundamental vital functions; each is more generalized. Finally, there is the great world of "lower" unicellular organisms in which all of the fundamental vital functions are performed by a single cell independently of all others. Such unicellular organisms represent physiological generalization *par excellence*, and some are, paradoxically, the most complex of all living cells and at the same time the simplest of living organisms (Fig. 3). Being single cells they are minute, and as the smallest of the living things treated in the following pages they have a special importance in many eco-

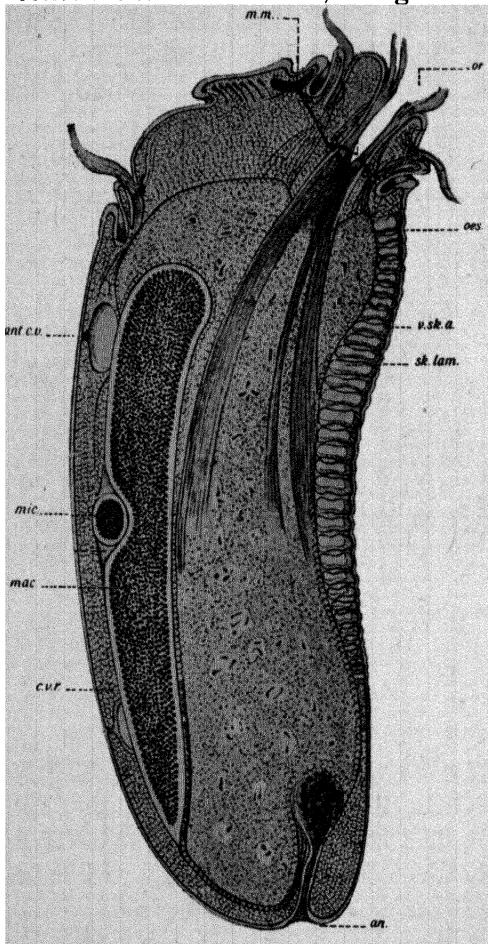


Fig. 3—*DIPLODINIUM ECAUDATUM*

A complex, ciliated one-celled protozoan parasite of the horse's stomach. Sharpe, who first described it, named 48 different parts of which the most important are: *or*, the mouth; *ocs*, the gullet; *sk*, skeletal elements; *m.m.*, motorium; *an*, the anal opening; *c.v.*, the contractile vacuole; *mac*, macronucleus, and *mic*, micronucleus

*Magnification, 920*

nomic, hygienic, and theoretical matters connected with human life, and at the same time they are of vast importance in aiding us to understand the principles of biological science.

### GROWTH OF KNOWLEDGE OF MICROSCOPIC ORGANISMS

Our knowledge concerning these minute creatures has grown step by step with the improvements made in the compound microscope. Toward the end of the seventeenth century the Dutch naturalist, Antonius van Leeuwenhoek, fastened a simple but

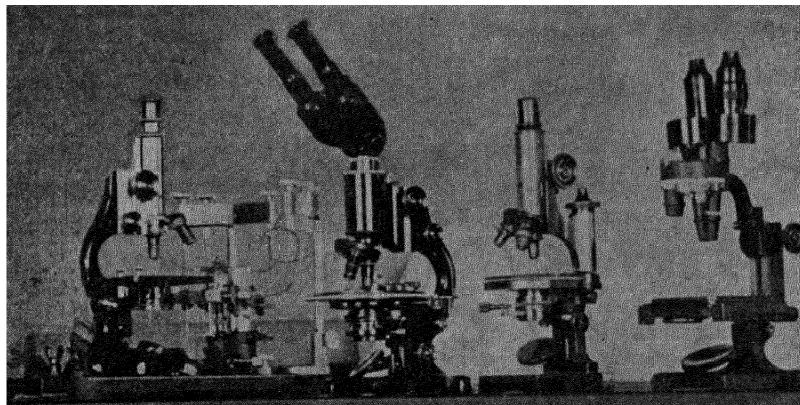


Fig. 4—MODERN MICROSCOPES IN USE BY THE AUTHOR

*Right to left:* (1) a low-power binocular microscope, useful in searching over a large field; (2) a monocular microscope for general work; (3) a microscope with special binocular attachment, used for high-power work; (4) a monocular microscope with special micro-dissection apparatus

powerful lens on a standard and thus made a simple microscope. Credit for the invention of the microscope is usually given to Galileo, but it was Leeuwenhoek who was the first to use a microscope in biological investigation and credit must be given to him for his discovery, in 1675, of the world of microscopic life. Modern microscopes have evolved from these primitive instruments into more and more powerful tools for seeing into the invisible. The advances have been in the technical perfection of lens-making, and for giving precision and clearness of vision as well as magnification (Fig. 4). It is amazing to see what biologists were able to do with the imperfect microscopes of one hundred years ago, and still more amazing to see what Leeuwenhoek did with the simple lens system of

his own creation. Some specimens of these crude instruments are in the British Museum. Yet with them Leeuwenhoek discovered minute living things in rain water which had been left exposed to air and sunlight. Some twenty-seven different kinds of protozoa were described by him more or less adequately, usually less, for today we are certain of only one of his types—*Vorticella* (Fig. 5). The microscopic organisms he observed were included by him under the comprehensive term “animalcula.” Thousands of different types of microscopic forms have since been added to Leeuwenhoek’s collection and are today classified as Bacteria, Algae, Protozoa, Rotifera, Worms, and Crustacea—many of them representatives of higher animals.

With this discovery in 1675 of the smallest living things came a renewal of the belief in spontaneous generation, and with regard to these minute organisms even the best scientific minds wavered. It required two hundred years more to prove that these microscopic organisms do not arise from lifeless matter and that each one comes from an organism like itself. To the biologist these minute living things are not only interesting in themselves, but their study throws a flood of light on some fundamental problems of biology and it is with this in mind that the present volume is written.

#### OVER A HUNDRED THOUSAND SPECIES OF MICROSCOPIC FORMS

There are upwards of 100,000 different species of these microscopic forms, every species comprising representative living organisms, each complete in itself. In some cases a particular interest marks out one type from the rest, but for the most part and from the point of view of the present volume, interest centers in the fact that *in these minute organisms we have bits of living matter which perform all of the vital activities performed by the highest types of animals and plants. A complete knowledge of any one of these living things would solve the riddle of all life.* The relative simplicity of the structures by which these vital activities are performed make them relatively simple to analyze: In the present treatment I shall make no attempt to catalogue the microscopic forms, but I will try to give an adequate idea of their general types of structure and of the part they play in nature.



Fig. 5—A PROTOZOAN COLONY OF BELL-ANIMALCULES  
(*Vorticella campanula*)

Each individual is anchored by a delicate thread of protoplasm which contracts spirally when the owner is disturbed. Highly magnified strands of pond scum (*Spirogyra*) are conspicuous, spiral chlorophyll structures showing through the transparent, tubular walls. Two strands are forming spores, being connected by ladder-like rungs in the process

## CHAPTER II

### FILTERABLE VIRUSES

#### TOO MINUTE FOR THE MOST POWERFUL LENSES

THE very smallest of living things are apparently too minute to be identified as individual organisms, even with the most powerful lenses. These, known as the "ultra-microscopic organisms," also as the "filterable viruses," cannot be seen, but are known only through their activities as causative agents of different diseases; as such their full consideration is not within the scope of this volume.

The filterable viruses derive their name from their ability to pass through porcelain or diatomaceous earth filters, the latter made of the silicious shells of diatoms.\* Our knowledge of filterable viruses is very limited and certain authorities have even questioned whether these minute particles are endowed with life. They seem in some way to be related to the formation of various cellular and nuclear inclusions,<sup>†</sup> and are very definitely the cause of diseases, not only in man but also in fishes, fowls, insects, and plants.

#### THEIR ACTIVITIES

Knowledge of these filterable viruses is a fairly recent development. Although vague references to them had been made before, it was only in 1892 that the Russian botanist, Dimitri Iwanowski, found that the leaf mosaic disease of tobacco was caused by a filterable infecting agent. After that the progress was rapid. In 1898 a causative virus of filterable nature was

\* Diatom (from the Greek, meaning "cut in two") is any microscopic one-celled alga belonging to the order Bacillariales; diatoms are remarkable for a silicified cell wall which persists as a skeleton after the death of the organism and forms kieselguhr. Diatoms are always found on submerged objects (wood, stones, etc.) to which they impart a slimy feeling. Kieselguhr is a fine, usually white, powder, and is used as an absorbent in dynamite and as a polishing material.

† An inclusion is a foreign body, usually of minute size, inclosed in a larger mass. In biology, it is a foreign body inclosed in a cell.

demonstrated for foot-and-mouth disease of cattle—a malady which had caused great losses to cattle raisers in our West. Among the diseases of man which are thought to be due to virus infections of this nature are smallpox, measles, epidemic influenza, rabies, psittacosis,\* and common colds. Among animals, in addition to the foot-and-mouth disease of cattle mentioned above, the various kinds of pox of sheep, the distemper of dogs, and the sac-brood disease of bees are said to be due to them. In plants the great group of mosaic diseases which infest tobacco, potatoes, and sugar-cane are worthy of note. The whole subject of filterable viruses is still in its infancy, but with continued research much valuable knowledge and a clearer understanding of their nature is sure to result.

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\* Psittacosis is a contagious disease of parrots which is communicable to man; the symptoms in man are those of a severe form of pneumonia.

## CHAPTER III

### BACTERIA

#### NEITHER ANIMALS NOR PLANTS

**A** N UNSIGHTLY scum-covered pool, a green ditch, or a more pleasing pond bordered by lily pads, may be, and usually is, the home of countless millions of the smallest living things. A pint fruit-jar filled from such a source furnishes endless delight to a microscopist, and to him who has patience combined with vision it contains all of the major secrets of life. For the most part these living things are minute, but they vary in size from the limits of vision with the highest powers of the microscope to forms which are just visible to the unaided eye.\* Some of the

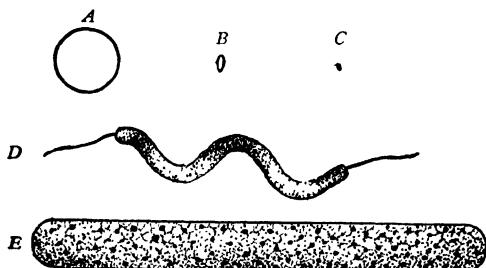


Fig. 6—RELATIVE SIZES

*A*, a human red blood corpuscle; *B*, a typhoid bacillus; *C*, an influenza bacillus; *D*, a common spirillum of stagnant water; *E*, a giant bacillus from the intestine of a centipede.

*From a drawing by the author*

smallest forms visible with the microscope are bacteria, some of which are tiny spheres known as cocci, some motionless, others moving with microscopic speed through the water. Others, cylindrical in shape, move across the field in a straight or zigzag

\* Measuring these small things is relatively simple, and there are many ways to do it. One simple method is to project, on paper, with the aid of a camera lucida, a scale of one millimeter divided into hundredths. The object to be measured is then similarly projected over the scale and the dimensions read. For greater accuracy one of the hundred divisions of the magnified scale may be divided into ten equal parts, each of such parts thus representing one-thousandth of a millimeter. This is called a *micron* and is usually accepted as the unit of microscopical size. It is designated by the Greek letter  $\mu$  (mu), corresponding to our English *m*. A human red blood corpuscle, which is a convenient object for comparing the sizes of very minute things, measures 6.9  $\mu$  in diameter and an influenza bacillus 0.5  $\mu$  to 0.2  $\mu$ . A millimeter is approximately one twenty-fifth of an inch, and a micron, one twenty-five thousandths of an inch.

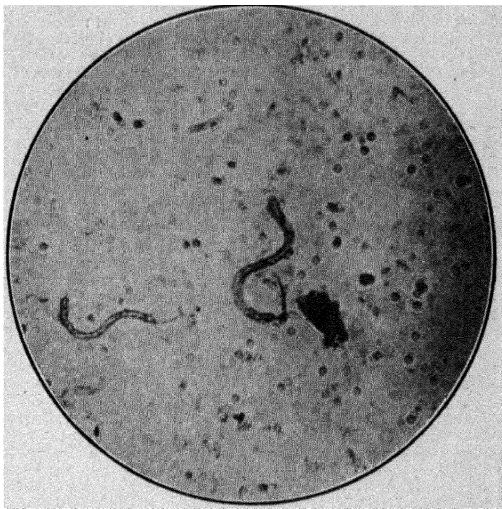
path, either alone—a bacillus—or in chains of bacilli somewhat like chains of miniature sausages. A curious spiral form—spirillum—like an animated corkscrew, darts about, now forward, now back, and turns on its long axis at the same time (Fig. 7).

These small living things—cocci, bacilli, spirilla—represent

Fig. 7—SPIRAL BACTERIA  
(SPIRILLUM UNDULANS)

Two individuals from stagnant water. A so-called flagellum may be seen at each end, and granules at intervals down the body; note the absence of a nucleus

From a photomicrograph by the author  
Magnification, 755



the living world of bacteria, creatures which can not be regarded as animals nor as plants, but which play an amazing rôle in nature. The science of bacteriology, developed in the last third of the nineteenth century on the foundations laid by Louis Pasteur (1822-1895) in France and by Robert Koch (1843-1910) in Germany, deals exclusively with these organisms. The structure and form of the hundreds of types that are now known differ little from the cocci, bacilli, or spirilla. All bacteria resemble one, or some slight variation of one, of these three types. The individual unit counts for very little to the student of bacteriology. The bacteriologist is concerned rather with the *mode* of its growth, the *substances* on which it can flourish, and with the *effects* of its activities. It is by these distinguishing characteristics that he describes bacteria. For the most part a single bacterium is too small to be studied effectively even with the highest power of a microscope. Culture methods, or means of rearing bacteria in mass, are, therefore, absolutely essential for the study of bacteria.

## THE MULTIPLICATION OF BACTERIA

Bacteria raised in a proper nutrient medium reproduce rapidly by simple cross division, whereby one bacterium splits into two, and, by a continuation of the process, in an incredibly short time great masses known as *colonies* are formed. "It has been esti-

*Courtesy of Dr. Edwin O. Jordan*

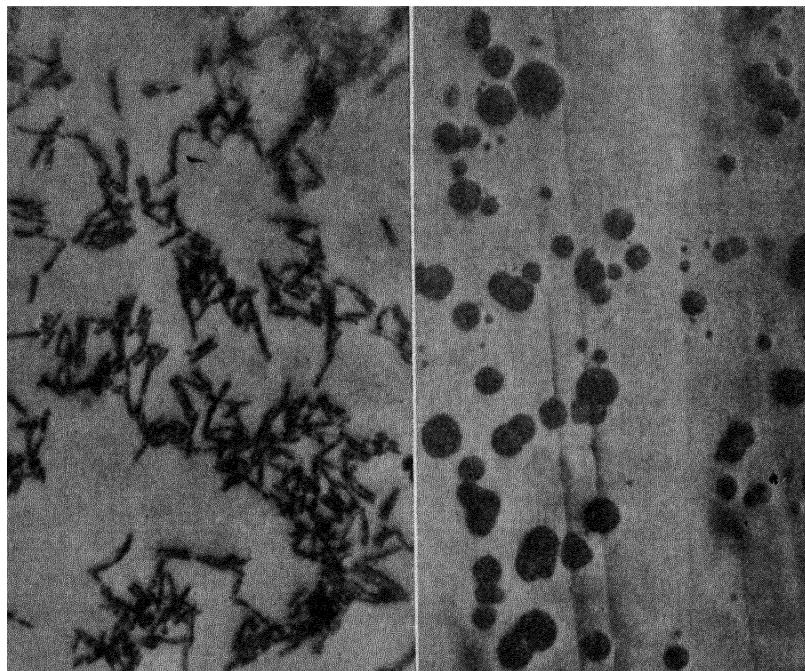


Fig. 8—THE BACILLUS WHICH CAUSES DIPHTHERIA

*Left*, individual cells—magnification, 1200; *right*, colonies grown on artificial medium—slightly magnified

*From photomicrographs*

mated that if bacterial multiplication went on unchecked, and the division of each cell took place as often as once an hour, the descendants of each individual would in two days number 281,500,000,000, and that in three days the progeny of a single cell would balance 148,356 hundred weight!"\* As a result of

\* E. O. Jordan, *General Bacteriology*. Such geometrical propagation is possible, however, only in theory, for their own secretion products, enemies of one kind or another, varying conditions of food, temperature, and culture medium, all tend to check their multiplication.

division the two new cells usually separate but in many types they remain together, thus forming colonies (Fig. 8) of one sort or another. If a coccus divides only in one plane, a chain of cocci is formed (streptococci); if it divides in two planes, flat sheets or masses are formed (staphylococci); if the divisions are in three planes, cubical aggregates (sarcinae) result.

### MASS ACTION OF BACTERIA

Obviously a single bacterium can not produce much of an effect good or bad, but where colonies of millions of them are involved their effects become very evident, and are beneficial or harmful according to the kind of the bacterial colony and its products. Thus in a can of improperly prepared vegetables or fish, the bacterium causing ptomaine poisoning in man will multiply, although deprived of free oxygen, and the excretion product of the multitudes of bacteria which result constitutes one of the most powerful poisons known. In a similar way many types of bacteria find a suitable environment in the organs and the cavities of the human body, and the products of their activity, known as toxins, produce various types of disease. Each toxin is different from every other toxin and is specific in its effects. *Streptococcus pneumoniae* causes about 95 percent of all lobar pneumonias; *Bacillus influenzae* causes influenza; *Bacillus typhosus*, typhoid fever; *Bacillus tuberculosis*, pulmonary tuberculosis; etc.

### HELPFUL BACTERIA

The mass action of bacteria, however, is by no means always harmful, indeed the beneficial effects of most forms far outweigh the deleterious effects of the injurious species. The natural decomposition of the dead bodies of animals and plants is brought about mainly by bacteria of decay and many of the products of their activities, usually accompanied by the production of malodorous compounds, are often useful to other living things. They also bring about the decomposition of protein to form relatively simple nitrogenous compounds, such as amides, peptones, and aromatic bodies, which are used directly as food by many types of minute saprozoic animals and saprophytic plants.\* Other products of this bacterial activity escape as gases—carbon di-

\* See "The Plant World," page 64, in this *Series*.

oxide, marsh gas, sulphuretted hydrogen, etc. Nitrogen is an element absolutely necessary for living things to synthesize (build up) more protoplasm. It is only available to animals when taken into the digestive cavity in the form of proteins derived from other animals or from plants. Through the putrefactive agency of bacteria some of the nitrogen-holding substances of dead animal and plant protoplasm become available as food for other types of pond-dwelling or earth-dwelling organisms. In this respect the putrefactive bacteria play much the same rôle as do the digestive ferments in the intestines of higher types of animals. Here, through ferment action, protein food substances are broken down by hydrolysis,\* to nitrogen-holding polypeptides and amino acids, simple substances which are capable of being utilized as foods by the cells of the body.

Although most of the all-important nitrogen in dead bodies of animals and plants is thus made useful to living organisms by

the putrefactive bacteria, some nitrogen escapes in gaseous form, usually ammonia ( $\text{NH}_3$ ), into the atmosphere. Animals can obtain their nitrogen only from animal or plant proteins, which they take in as food. The great majority of plants, including all the higher plants, can get their nitrogen in the form of salts (nitrates chiefly), which, when dissolved in water, are absorbed

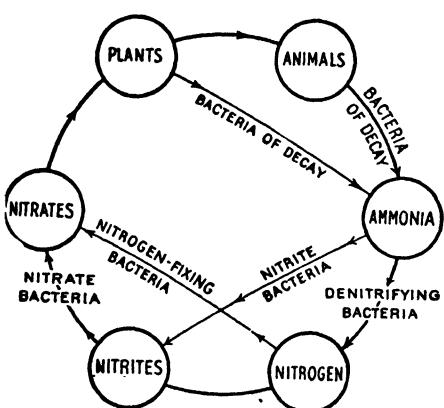


Fig. 9—THE NITROGEN CYCLE

by the roots. Very few plants have the power to utilize the free nitrogen which constitutes a large percentage of the air, but certain types of bacteria—known as the “nitrogen-fixing” bacteria—have the ability to do this and they play the part of a middleman between the nitrogen of the air and the living protoplasm. Thus certain forms of bacteria (*Azotobacter*) found in the soil actually use the nitrogen of the air as food to build up

\* Hydrolysis is a chemical process in molecules of a substance whereby, by the addition of water, the substance is split into simpler substances.

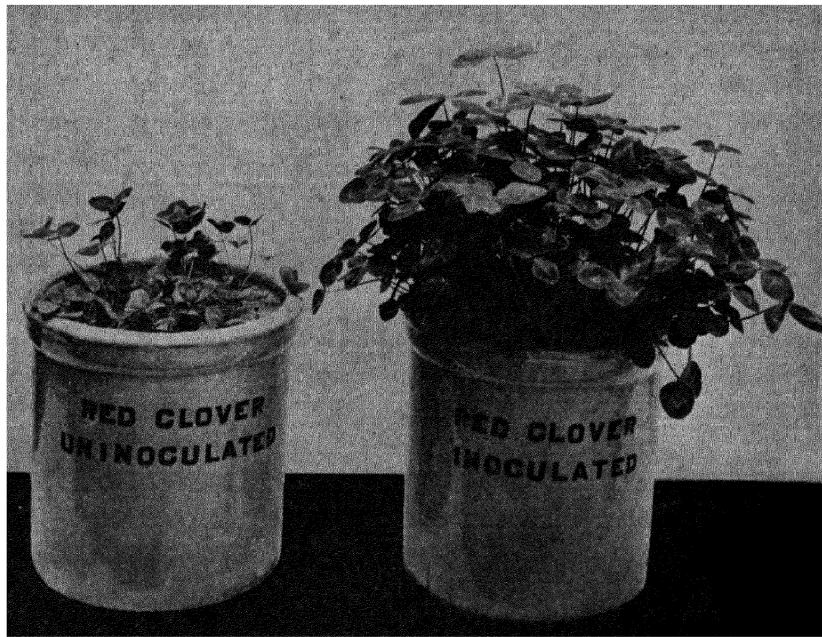


Fig. 10—BACTERIA ARE USED TO STIMULATE CROP GROWTH

A comparison between a red clover plant which has been inoculated with the proper nodule-forming bacteria and another plant of the same species which has not been inoculated. *From a photograph by the Department of Agricultural Bacteriology, University of Wisconsin*

their own protoplasm. Root nodules on certain leguminous plants contain colonies of another bacillus (*Bacillus radicicola*), which likewise obtains its nitrogen directly from the air. This useful function of these nitrogen-fixing bacteria has been turned to good account in agriculture. Not only is "green manuring," or plowing in of leguminous crops (rye, clover, vetch, cow peas, etc.), commonly practiced, but the marketing of pure cultures of nodule-forming bacteria under different trade names is now common.

Another form of bacteria, known as the denitrifying bacteria, perform the function, perhaps not so useful, of converting some of the ammonia into free nitrogen once more (Fig. 9).

Other types of bacteria (such as *Nitrosomonas* and *Nitroso-coccus*) are causative agents in the oxidation of free ammonia to nitrous acids and nitrites, salts that are not available for plants, but the bacillus *Nitrobacter* completes the oxidation by

bringing about the transformation of nitrous acid to nitric acid and nitrates.

#### MAN'S CONTROL OF BACTERIA

Man has learned how to control this mass action of bacteria in many ways and to turn it to good account in his economic and commercial life. Not only has he devised ways to prevent bacteria from spreading by means of his public hygienic methods and by private prophylaxis, but he has also devised ways to combat the diseases they cause by utilizing the various protective substances that the body develops to overcome infection. Inoculation\* and vaccination\* are to-day used as protective measures in many diseases. By these methods the body is stimulated to produce antitoxins and antibodies to overcome the ravages of the bacteria, and to induce immunity or resistance to disease.

In industry the activities of many kinds of bacteria have been harnessed. Thus the "curing" of tobacco whereby the special flavoring is obtained, the ripening, with distinctive tastes, of beers and wines, the making of many different kinds of cheese, the flavoring of butter, etc.—all are due in large part to the controlled action of bacteria. Similarly, the retting of flax and the tanning of hides are influenced at many points by bacteria.

In the disposal of sewage, obviously of the greatest importance for wholesome life in cities, the major part of the treatment of the waste material is performed by bacteria of decay and by the anaërobic bacteria—those bacteria which can live without the presence of free oxygen. For localities without sewerage systems, the modern "septic tank," which utilizes these principles in its construction, has come as a boon for sanitation.

A great world of practical importance—all dealt with in the science of bacteriology—is thus revealed by the study of the ordinary coccus, bacillus, and spirillum of stagnant water. Thanks to these small creatures other forms of stagnant pool organisms—desmids, diatoms, filamentous algae, etc., among plants, and protozoa, rotifers, crustacea, worms, etc., among animals—are able to live and thrive, for without the work of the bacteria the continuous interchange of materials between the organic and the inorganic worlds would be impossible.

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\* See the Glossary of this book, pages 121 and 124.

## CHAPTER IV

# SPIROCHAETES

### FORMS CLOSELY RELATED TO BACTERIA

**I**N HIS great work on *Die Infusionsthierschen als vollkommne Organismen (Infusoria as Complete Organisms)*, published in 1838, a famous German microscopist, Christian Gottfried Ehrenberg, described the genus of bacteria known as spirillum as a rigid, corkscrew-like organism, and the genus known as spirochaeta as a flexible, undulating, spirally wound organism. His type species of the latter—*Spirochaeta plicatilis*—is a sinuous, somewhat snake-like form found among decomposing matter in pools and ditches. The difference between these two genera—spirillum and spirochaeta—is slight at best according to Ehrenberg's characterizations. In many of the species discovered since, it is difficult to decide whether an individual is rigid or not. In any case, the spirochaetes, as the group is called, are closely related to spirillum and through this genus to the bacteria, but are usually treated as a distinct group of minute organisms. Some authorities regard them as related to the flagellated protozoa,\* but the majority of observers consider them as intermediate forms between bacteria and protozoa.

### THEIR IMPORTANCE

No matter how scientists may differ as to their classification, spirochaetes are organisms which cannot be neglected, for they are responsible for some of the most loathsome diseases known to man. The majority of species, however, are probably harmless and live as commensals<sup>†</sup>—harmless or perhaps even useful inhabitants—in the digestive tracts of all animals, or as free organisms in natural waters. They differ sufficiently from each other to make it possible to classify them to some extent as be-

\* See "The Animal World," page 39, in this Series.

† Commensal (from two Latin words, meaning "together at table"); in biology an organism, not truly parasitic, which lives in, with, or on, another, partaking usually of the same food; both species may be benefited by the association.

longing to different genera, under the names of *Spirochaeta*, *Saprospira*, *Cristispira*, and *Treponema*, the last of which is sufficiently diverse to have been split into a number of sub-genera by different observers.

The first two of these genera are not parasitic, but may be found in stagnating fresh and salt waters. They have no particular importance to man, but probably play some part in causing decay of dead animal and plant matter.

#### TYPES AND SPECIES

*Spirochaeta plicatilis* is not uncommon and gives the impression of an actively undulating microscopic snake (Fig. 11). It is distinguished by the possession of an axial bar, called a columella, which runs from one end to the other, and around this

Fig. 11—*SPIROCHAETA PLICATILIS*

An inhabitant of polluted water. Only part of a specimen is shown in this drawing made by the author

From a photomicrograph by Marguerite Zuelzer  
Magnification, 1500

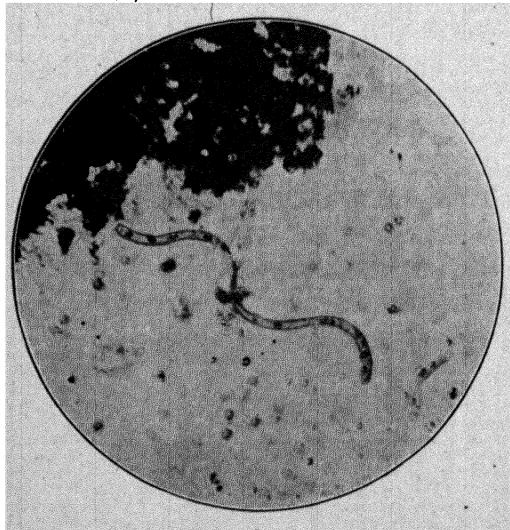


Fig. 12—*SAPROSPIRA*

A harmless non-parasitic spirochaete from stagnant water. The granules down the body are like bacteria spores, but are called "coccoid" bodies

From a photomicrograph by the author  
Magnification, 800

bar the protoplasmic body coils like a spiral staircase about its newel post. It shows very few other structures, and no nuclear differentiation or specializations can be distinguished in it beyond the presence of very fine granules, known as chromatoid granules,

similar to the chromatin granules found in ordinary cell nuclei.\* The organisms are about  $5\mu$  thick and vary in length from  $200\mu$  to  $500\mu$ .

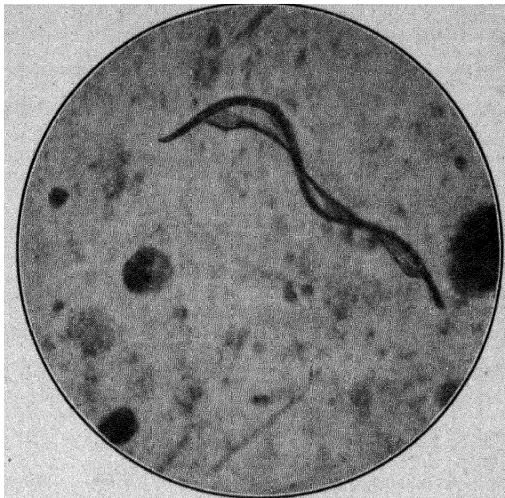


Fig. 13—*CRISTISPIRA  
BALBIANII*

A large spirochaete from the digestive tract of an oyster. The "crista," or winding flange-like shelf, is not an undulating membrane but is rigid.

From a photomicrograph by the author  
Magnification, 1000

Another free-living but much larger type, *Saprospira* (Fig. 12), is also found in polluted waters. It is several times thicker than spirochaeta and resembles certain types of algae except for the absence of chlorophyll.† Reproduction of this form is normally by transverse division, but it also reproduces by the formation of so-called "coccoid bodies" which are analogous to the spores of bacteria. Like the latter, *saprospira* is made up of an aggregation of the internal chromatoid granules. There is no columella, and no differentiations of any kind are apparent.

Another type of spirochaete, *Cristispira* (Fig. 13), differs from the two types described above, in having a definite spirally-wound flange-like shelf running the length of the body. These forms are never injurious to man; they are present as parasites in practically all oysters and clams, but they do not cause any apparent harm. The several species of this genus, as well as all species of the next genus, *Treponema*, are parasitic forms—some harmless and some pathogenic. *Cristispira* was discovered in 1882 by A. Certes in one of the organs of the oyster in which it lives. Practically every oyster, if examined immediately after removal from the water, can be found to harbor this spirochaete. It is smaller than *Saprospira* and differs from *Spirochaeta* in

\* See "Heredity and Variation," page 44, in this Series.

† See "The Plant World," page 13, in this Series.

having whorls of greater amplitude though much fewer in number. Its chief characteristic is the presence of a relatively wide, rigid ledge, known as the *crista*, which runs spirally about the body from end to end and which is thought to correspond to the columella characteristic of Spirochaeta. Like *Saprospira* it reproduces by transverse division and by coccoid bodies.

### CAUSE DISEASE IN MAN AND BEAST

The smallest examples of the spirochaetes—the *Treponema* type—are frequently harmful parasites and are frequent causes of disease in man and domesticated animals. Relapsing fevers, tick fever, yellow fever, infectious jaundice, rat-bite fever, venereal diseases of man and other mammals, and blood diseases of birds, are due to one or another form of *Treponema*.

The species of this genus are all minute (from  $2\mu$  to  $30\mu$ ) and are so thin and delicate that, for the most part, they can be seen only with difficulty (Fig. 14). This may be realized by

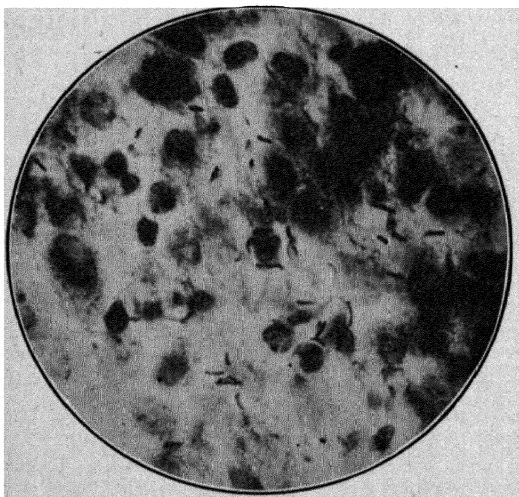


Fig. 14—*TREPONEMA MICROGYRATA*

A parasitic spirochaete which lives in diseased tissues of mice. The turns of the spirals are so close together that they can barely be recognized

From a photomicrograph by the author  
Magnification, 1000

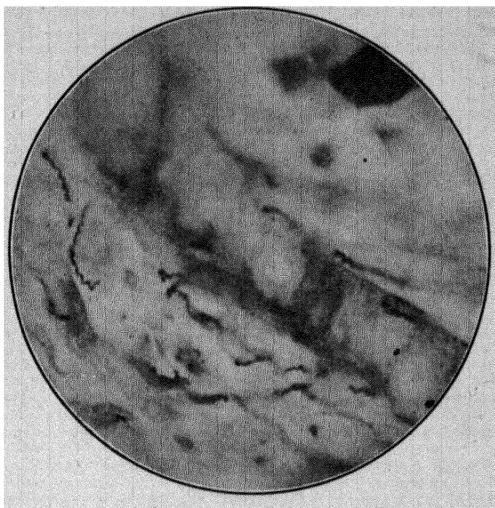
the fact that the minute organism, which is the cause of syphilis, was not seen until 1905, in which year Fritz Schaudinn, a German and one of the keenest observers among modern zoologists, discovered it and named it *Spirochaeta pallida*, a name which he later changed to *Treponema pallidum* (Fig. 15). Prior to this, however, somewhat similar spirochaetes had been observed and identified as *Spirillum*; one, named *Treponema recurrentis*, was

interpreted as the cause of relapsing fever in Europe and another named *Treponema duttoni*, was identified as the cause of "tick fever" in Africa. Relapsing fevers of other localities have

Fig. 15—*TREPONEMA PALLIDUM*

The cause of much human suffering. These pernicious spirochaetes have the power of invading human tissues of all kinds. The organisms are very delicate, but after silver nitrate impregnation they are easily seen

From a photomicrograph by the author  
Magnification, 1000



since been attributed to different species of spirochaetes, variously called *Spirosoma*, *Spiroschaudinnia*, again *Treponema*, or even *Spirochaeta*.

### THE SPREAD OF SPIROCHAETE DISEASES

In many diseases caused by these minute spirochaetes the infection is passed from individual to individual by contact. Such is the case, for example, in syphilis which is universally distributed, or in the African skin disease known as yaws or pian or framboesia (caused by *Treponema schaudinni*) and discovered by the Italian-British pathologist, Aldo Castellani (1875-

), a well-known student of tropical diseases. In other types of spirochaete diseases, however, the human victim does not transmit the organisms directly to others, but infects some blood-sucking insect, *e.g.*, a mite or a tick, in which the spirochaetes live and multiply. These animals in turn pass the spirochaetes to other humans. The discovery of arthropod-borne diseases was one of the greatest discoveries in the history of preventive medicine. It was first made by American pathologists, Theobold Smith (1859- ) and several collaborators, in connection with a blood disease of Texas cattle, variously called Texas

fever, tick fever, or bovine malaria. The cause of Texas fever is not a spirochaete but another type of blood parasite called *Babesia bigemina* and is related to the organism causing malaria in man. The transmission was found to be due to a tick which lives in the hair of cattle.

In connection with spirochaete-transmission of disease two physicians, Dr. Joseph Everett Dutton (1876-1905) of England and Dr. John L. Todd (1876- ) of Canada, discovered in 1903-1904, while on an expedition for the Liverpool School of Medicine, the cause and mode of transmission of an African disease which the natives called tick fever. The spirochaete which is responsible for this type of relapsing fever was named *Treponema duttoni* in honor of one of the discoverers who lost his life during the investigation. It was found that the tick (*Ornithodoros moubata*) abounds in old mattresses, clothing, etc., left in the caravan camping sites and is ready to infect individuals of the next caravan. It was also found that every organ of the tick becomes penetrated with spirochaetes, whereas in man they remain in the blood. The parasites may continue to live in the tick for as long as eighteen months after a single infection.

There is reason to believe that relapsing fever in Europe is transmitted by human body-lice and head-lice. Bedbugs may also be carriers of infection. It has long been known that uncleanliness, crowding, and close contact with infected persons are characteristic conditions leading to the spread of the disease. Relapsing fever has been produced in monkeys by inoculating them with crushed infected lice. It is possible that in humans the infection is transmitted when the fingers in scratching crush infected lice.

Yellow fever and infectious jaundice are spirochaete diseases which are transmitted, not by ticks, but by mosquitoes, and the history of the discovery of this relationship is the history of heroes and martyrs to the glory of American medicine. Many of us today remember when yellow fever frequently flared up in epidemic form in our southern States, and many of us recall the types of quarantine set up at such times. Express packages, mail, laundry, etc., from a yellow-fever locality underwent sterilization before delivery in another State. Today such precautions are known to be without value and a needless expense.

The yellow-fever commission sent by the United States to Cuba in 1900 was perhaps the best result of the Cuban war. The commission consisted of Dr. Walter Reed, Dr. James Carroll, Dr. Jesse Lazear, and Dr. A. Agramonte. Dr. Lazear died

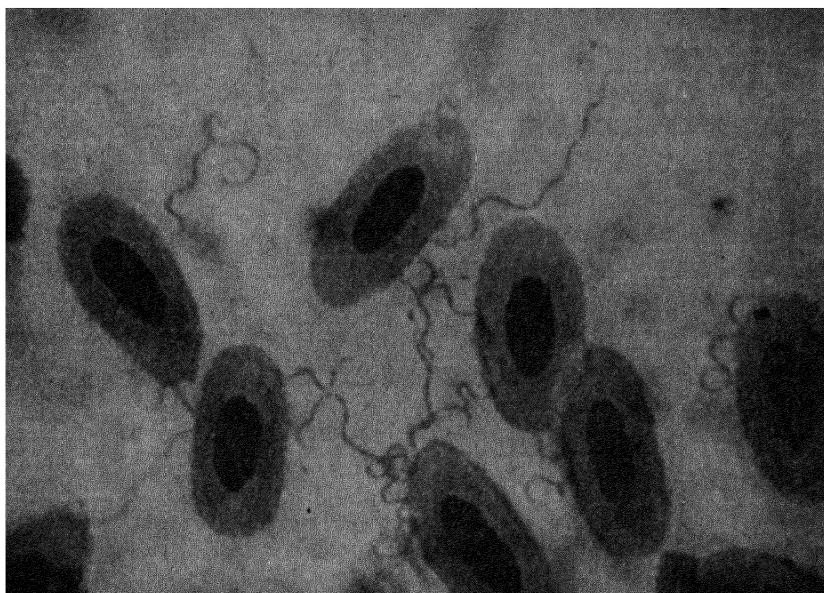


Fig. 16—*TREPONEMA GALLINARUM*

A spirochete which causes relapsing fever in ducks and other fowls. Similar microscopic snakes cause relapsing fever in humans. The elliptical-shaped bodies are blood cells

*From a photomicrograph by the author*

*Magnification, 1500*

of yellow fever and Dr. Carroll contracted it and lay at the point of death for several days, but finally recovered. They established the fact that exposures to the disease or to soiled articles, bedding, etc., from its victims, are without effect, but the bite of a single infected mosquito, even under the most sanitary conditions, produces the disease. They learned that when a mosquito has bitten a yellow fever victim and has become infected, it cannot transmit the disease for a period of twelve days—in other words a period of development in the mosquito is necessary. They learned that a human being, bitten by an infected mosquito, will infect another mosquito only during the first three

or four days of the disease. It was further established that once a mosquito was infected the infection was for the lifetime of the mosquito. The ability to transmit yellow fever is practically limited to one species of mosquito, *Aedes (Stegomyia) fasciatus*, which has a world-wide distribution but breeds only in the tropics and the warmer parts of the temperate zones.

The entire field of yellow fever prophylaxis became known long before the organism causing the disease was seen or its type known. It was supposed to be due to one of the filterable viruses until the Japanese bacteriologist, Hideyo Noguchi, who lost his life in Africa while studying an African type of yellow fever, demonstrated in 1919 a spirochaete as its cause. This he named *Leptospira interrogans*, distinguishing it from other spirochaetes by the hook-like turn of the spiral at one end, and associating with it the cause of another human disease, infectious jaundice (Weil's disease), under the name of *Leptospira icterohemorrhagiae*. These two types differ so little from other spirochaetes that authorities generally include them with *Treponema*.

Similar blood diseases, due to the spirochaete *Treponema galinarum* (Fig. 16), are found in ducks and in fowls generally. Their transmission is brought about from bird to bird by a tick, called *Argas miniatis*, living in the feathers of the bird.

It is hardly possible to draw a line between these blood-dwelling spirochaetes, which by some authors are called *Spirosoma*, and the types which invade other tissues or the true treponemas. The blood-dwelling forms of *Treponema duttoni* in human blood become tissue-invading forms in the tick, and similar combinations of mode of life are characteristic of the majority of pathogenic types. So it is with *Treponema pallidum*, the cause of syphilis (Fig. 15), with *Treponema (Leptospira) icterohemorrhagiae*, the cause of infectious jaundice, and *Treponema (Leptospira) interrogans*, said by Noguchi to be the cause of yellow fever.

The life histories of the treponemas are still matters of controversy. In these minute forms some observers maintain, others deny the existence of reproductive ("coccoid") bodies such as are found in other spirochaetes. The fact that in some cases the virus cannot be filtered out lends support to the view that some such minute germs capable of transmitting the diseases must be formed.

## CHAPTER V

# THE FUNDAMENTAL, LIVING SUBSTANCE— PROTOPLASM

### DIFFICULT TO COMPREHEND

**L**IVING substance—protoplasm—appears to be a difficult concept for the uninitiated to comprehend. “Living” things are all about us, but ordinarily we do not see protoplasm—what we do see is the lifeless external skin, or covering, of an animal, or the equally lifeless bark of a tree or other coverings of vegetable life, all formed through the activities of protoplasm, but not themselves the living protoplasm. With the microscope, however, it is possible to see and to study not only living things as organisms but also the protoplasm of which they are composed. This is particularly illuminating when observed in the smallest living unicellular organisms. So far as size is concerned, it must be clearly understood that many multicellular animals (*Metazoa*) and many multicellular plants (*Metaphyta*) are microscopic, but the limits of the present volume forbid a discussion of these.

### A TYPICAL CELL

A typical cell\* is made up of protoplasm and its derivatives, and, structurally, consists of a cell body (cytoplasm) and a differentiated portion called the nucleus. The cytoplasm contains inclusions of many kinds, some of which, known as plastids (*e.g.*, chloroplastids, pyrenoids, etc.), are functional in protoplasmic activities, *i.e.*, they perform some of the life processes,† whereas others, known as metaplastids, are products of protoplasmic activity (starch, paramylum,‡ fats, oils, etc.), and play but a passive rôle in the life of the cell.

\* See “The Coming and Evolution of Life,” page 8, and “The Plant World,” pages 5 and 34, both in this *Series*.

† See “The Coming and Evolution of Life,” page 7, in this *Series*.

‡ Paramylum is a carbohydrate, allied to starch.

The nucleus is absolutely essential for cellular activity; without it protoplasm soon becomes lifeless. This is readily demonstrated by cutting into pieces any large single-celled animal, such as an Amoeba\* proteus or a Stentor, so that some of the fragments are without a nucleus. Such pieces invariably die, while the piece which has a nucleus lives. The sizes, forms, and number of the nuclei in different cells vary widely. Some cells have no definitely formed nuclei but have the essential nuclear substances distributed throughout the cytoplasm. Such is the case in bacteria and spirochaetes, where no definite nucleus with its nuclear membrane and contained nuclear substance, called "chromatin," can be found. Chromatoid granules are distributed throughout the bodies of bacteria and spirochaetes and at times are segregated in dense masses to form the spores of bacteria and the "coccoid bodies" of spirochaetes. Thus these minute things do not have the same type of organization as do cells of more complex organisms, and in view of this fact, the English biologist, Dr. E. A. Minchin, in *An Introduction to the Study of Protozoa*, refers to them as organisms of a non-cellular grade.

### THE NATURE OF PROTOPLASM<sup>†</sup>

Physically, protoplasm is a glassy, sticky, fluid aggregate of substances, each of colloid (jelly-like) nature. Chemically, it is not a definite single substance but is a mixture of substances which are not in solution nor do they form a true chemical union. Their physical state is analogous to, but infinitely more complex than, an emulsion of oil and water. Protoplasm is denser than water and does not mix with it, but upon rupture of the surface membranes of a cell may be readily disintegrated in water.

Chemically, protoplasm is a combination of organic substances of complex nature—proteins, carbohydrates, and fats—together with many inorganic substances, such as salts of various kinds, water, and gases, such as carbon dioxide and oxygen. Of these, water is the most abundant component and subserves many important functions. It is essential for bringing about the breaking down of the compound salts into their constituent elements, thus setting these elements free to enter into new chemical com-

\* See "The Coming and Evolution of Life," page 9, figure G, in this Series.

† See "The Coming and Evolution of Life," page 10, in this Series.

binations. Water is also necessary for the continuance of those processes of metabolism, *i.e.*, the building up and the breaking down of proteins and carbohydrates, which are characteristic of living protoplasm. Salts usually found in protoplasm are the chlorides, carbonates and phosphates of sodium, potassium, ammonium, calcium, magnesium, and iron.

Of the organic compounds, the proteins are the most complex and the most variable, but they always contain some nitrogen and a carbohydrate. In the nucleus the proteins are particularly complex, especially in the substance known as chromatin, the material from which definite bodies called chromosomes\* are formed at periods of cell division (see Fig. 2, page 9). It is most remarkable that these chromosomes are always of the same number in a given species of plant or animal. They are the seat of the genes—those hypothetical genetic units which are commonly believed to bear the hereditary traits.

The carbohydrates and fats are combinations of carbon, hydrogen, and oxygen in which the hydrogen is always twice that of oxygen, just as in water. They are present in great variety in protoplasm. Those carbohydrates which are least easily broken down serve as the structural materials of cellulose (the covering of plant cells), of wood, etc., while others, less stable, are sources of energy for the organism—an energy which is made available through oxidation and by reducing agents generally. Fats have a much higher energy content, because their larger molecules have more carbon and hydrogen atoms capable of being oxidized. Their ability to unite with electrolytes<sup>†</sup> to form soap-like compounds with varying water-soluble properties is particularly significant.

### ORGANIZATION

These fundamental substances of protoplasm should be kept in mind if we wish to gain even a glimmer of the nature of vital processes. We speak of a certain combination of such substances as the *fundamental organization* of a species, and recognize the fact that no two such combinations are alike. The

\* See "Heredity and Variation" in this Series.

† An electrolyte is a compound substance which can be decomposed (separated) into its elements by an electric current. Salts, as a rule, disassociate readily, acids in various degrees. Substances which do not conduct an electric current are called nonelectrolytes.

differences, which, however, cannot be detected chemically, have to do, in all probability, with specific differences in the proteins. In the same environment a given fundamental organization invariably develops the same types of structures and in the same order, and such structures, coming from the fundamental organization are included in what we call the *derived organization* (see page 38). The derived organization of a species is seen in its best development in the adult organism. The fundamental and the derived organizations constitute the working parts of the infinitely complex aggregate which we call a living thing; they make up the "organization" of living matter, and such organizations are as varied as there are types of cells of animals and of plants.

### VITALITY

Vitality is the term used to express the *activity of the protoplasmic constituents of the organization*. The numerous substances found in protoplasm, be they temporary or permanent in nature, are constantly acting and reacting with one another and with other substances by which they are surrounded in the environment, in both chemical and physical fashion. Nevertheless, they must maintain a state of balanced equilibrium or be capable of quickly re-establishing an equilibrium; if otherwise, protoplasm would disintegrate. One means of maintaining this balance or equilibrium is the presence of living membranes, not only about the cell, but also about the nucleus and by lipoid (fat-like) membranes about the colloidal particles. These membranes regulate permeability (the passage of substances in and out), while the speed of chemical reactions is hastened by elusive chemical substances—the catalyzing and the synthesizing enzymes.\*

### ENCYSTMENT AND THE FUNDAMENTAL ORGANIZATION

The concepts outlined above—organization and vitality—representing the complicated and interrelated parts of an organism and the equally complicated and interrelated activities of those parts, give us a basis for an estimate of the nature of life,

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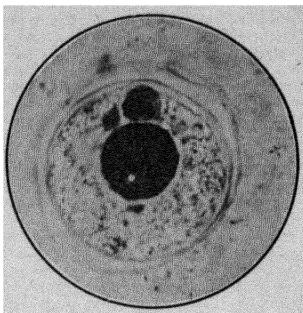
\* See the Glossary of this book, page 120.

but they do not tell us what life is. They also pave the way for an interpretation of a characteristic phenomenon in the life-history of the smallest living things. This phenomenon, known as *encystment* or the *encysted state*, is a period of rest in the life of a minute organism. When about to encyst, a unicellular

Fig. 17a—CYST OF UROLEPTUS MOBILIS

With protoplasmic membrane and cyst membrane. Encystment is complete and the organism may remain in this dried state for months or even years

From a photomicrograph by the author  
Magnification, 1000



individual becomes much condensed through loss of water; waste matters in the protoplasm are eliminated and motile organs and other structures characteristic of the active individual are absorbed or discarded, all these changes occurring before or during the secretion of a firm, resistant membrane or *cyst wall*, within which the cell is protected against adverse environmental conditions, such as drought. Within this cyst an individual may remain in a completely dried state for months or even years, but when again immersed in a proper medium it emerges from the cyst as a young, vigorous individual, typical of its own species (Figs. 17a and 17b).

Are such encysted cells *alive*? They are, but not in the same sense as the active, motile individuals which emerge from the cysts. I am accustomed to describe such an encysted form as comparable with an automobile in the garage, and the individual which has emerged from the cyst, with the machine under full headway. The organization of the car in the garage is perfect, but static. Introduce oxygen, gasoline, and a spark, and the parts of the organization work together harmoniously; the machine has become dynamic.

So it is with an encysted organism. Except for the absence of water, the organization of the protoplasm of the encysted individual may be considered perfect; yet without this water, interaction of the constituent substances is impossible, and the

organization is consequently static. But such static protoplasm has the possibility of activity when the proper condition is provided—when water is added it becomes dynamic. It is not

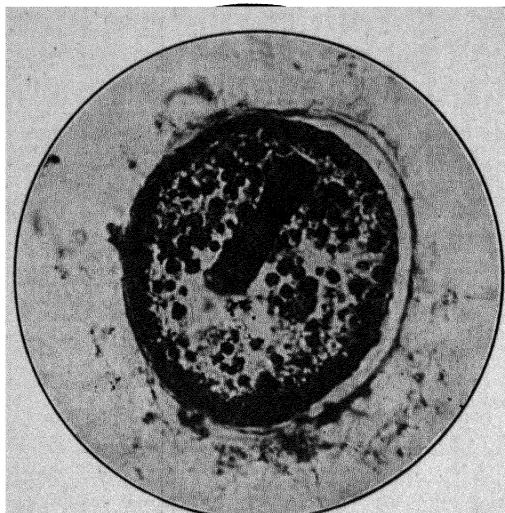


Fig. 17b—SECTION OF AN  
ENCYSTING SPECIMEN  
OF DIDINIUM  
NASUTUM

The old macronucleus has fragmented into many pieces, which are not yet absorbed

From a photomicrograph by the author  
Magnification, 1000

correct, therefore, to state that life is dynamic, or that life and activity are synonymous. Without organization, activity is impossible, but our encysted unicellular organism shows us that *organization is possible without activity*. Hence living matter may be regarded either as protoplasmic organization in action, or as organized protoplasm in a static condition with the possibility of action.

#### INDIVIDUALITY UNCHANGED BY ENCYSTMENT

Another point is illustrated by our encysted organism. Since all external structures characteristic of the active individual are lost with encystment (Figs. 17a and 17b) there are no visible structures to indicate what type of organism is represented by any cyst. Yet no two cysts are alike, although they may be indistinguishable to all but a specialist. If we watch individuals of *x* and *y* species encyst, and after some days or months, during which the *x* cysts cannot be distinguished from the *y* cysts, observe these organisms emerge, we find that *x* individuals never emerge as *y* but always as *x*, and *y* cysts always produce *y* individuals only. This means that the organizations of the *x* and the *y* cysts, although made up of protoplasms, which, when analyzed,

yield apparently the same substances, differ nevertheless from each other as much as the active  $x$  individual differs from the active  $y$  individual. The organization of the protoplasm of the two forms must be radically different. These differences of organization are supposed to lie in the molecular make-up of the proteins of which their protoplasms are composed.

In its normal medium an encysted organism emerges as an active individual of its own species, and nothing else can happen so long as the environment remains the same. Its protoplasmic organization as an adult, however, is different from that of its protoplasmic organization when encysted, and here is where our analogy with the automobile breaks down. The parts of an automobile, when active, are the same as the parts when static, but the parts (chemical substances) of an organized living thing undergo marked changes with the activities in which they take part. Thus when water and oxygen are introduced through the cyst membrane of an encysted *Uroleptus halseyi*, new structures — motile organs, cell mouth, and a different shape of the body — appear, which were not present before (Fig. 18). These new structures are due to changes brought about, presumably, by the oxidation of certain substances in the outer zone of the protoplasm of the encysted organism, and these changes are always the same for the same species. Oxidation results in the breakdown of such substances, and in the transformation of the latent energy of their combination into a dynamic form of energy expressed by movement. New substances are formed, some of which may be responsible for the motile organs or the various other structures of the adult. All of them, however, react with the other substances of the protoplasm, and so set up those connected chains of protoplasmic activities, destructive and con-



Fig. 18—THE DERIVED ORGANIZATION OF  
*UROLEPTUS HALSEYI*

A group of individuals showing little more than a chain of macronuclei and the general cigar shape of the body.

*From a photomicrograph by the author  
Magnification, 600*

structive, characteristic of life, which we group under the comprehensive term metabolism.

It thus happens that the protoplasmic organization of a living form is constantly changing in its active state with or without the formation of structures not previously present. If new structures are thus formed, such activity leads to successive states of complexity of organization which we include under the terms *development* and *differentiation*. With differentiation or development of new parts, a division of labor is set up among these parts; hence at each stage of its growth the organism has a slightly different organization accompanied by slightly different functions of its parts. Finally, when the possibility of further structural change is exhausted the individual is said to be fully developed.

#### DERIVED ORGANIZATION

We can distinguish, therefore, between the fundamental or initial organization of the protoplasm of an individual and the *derived organization* resulting from development. Encysted unicellular organisms and the fertilized egg-cells of all animals and plants represent protoplasm having the fundamental organization of the species in each case, no two of them being exactly alike. We must not consider the protoplasm of the adult to be identical with the protoplasm of its one-celled stage for it decidedly is not. Similarly, the millions of species of animals and plants represent protoplasm with its particular ultimate derived organization in each case, and no two of them are identical.

The higher many-celled plants, like animals, all start as single-celled fertilized eggs. The protoplasm in one such cell has the fundamental organization to develop into an adult, in which the protoplasm of the various cells, through specialization and differentiation, changes in organization—thus becoming the derived organization. In fact with the various delicate tests at the command of biologists, minute differences between individuals of the same species can be demonstrated.

The classification of animals and of plants, as given today, is based upon studies and comparisons of the final or derived organizations of the species. With increased knowledge it is hoped and highly probable that more attention will be given to

the fundamental organization. Students of heredity—geneticists—speak of the *genetic structure* of an organism as contrasted with its outward appearance.

### IRRITABILITY AND ADAPTATION

In every stage of development the substances making up the protoplasmic organization of an individual are constantly reacting with each other and with those of the environment.\* These activities are maintained in a delicately adjusted equilibrium which changes and readjusts itself with each stimulus from without. It is this unstable equilibrium, this sensitiveness, which is meant by the expression *irritability of protoplasm*. Irritability may be manifested by visible reactions, but it is probable that responses to stimuli are for the most part invisible since they have to do with activities and adjustments of the protoplasmic substances rather than with the response of the organism as a whole.

The same environmental stimuli acting upon the fundamental organization of a given species invariably produce the same reactions and therefore result in the same type of derived organization, so that the "species" remains constant. But if the environment be changed, then the stimuli from it become unusual, and the protoplasmic response becomes more or less different from the normal. If the change is too drastic, the protoplasmic substances are unable to adjust themselves to a new equilibrium and disintegration results. Thus heat will speed up and cold will retard activities, but too much heat coagulates the protoplasm. Chemicals of different kinds introduce novel types of stimuli to which the protoplasmic organization may adjust itself in such a way that a different type of derived organization or an *enduring modification* results, a change that is maintained so long as the source of the unusual stimuli remains in the environment.

Obviously any change in the fundamental organization would also result in a different series of reactions to the stimuli of the normal environment. Thus, when, in fertilization, two cells fuse, a new type of fundamental organization is produced which, with the usual normal stimuli, may or may not be indicated by structural peculiarities of the derived organization. Should the fun-

\* See "The Coming and Evolution of Life," page 6, in this Series.

damental protoplasm be changed drastically thereby, so that the derived organization also becomes modified as indicated by structural peculiarities, a new form may result. New types arising in this manner, if they breed true, are called *mutations*,\* and have the value of new species. It is this principle which is the basis of modern genetics, or the study of heredity and related subjects.

Adaptation to the environment is thus an outcome of the irritability of protoplasm. New species may originate, either (1) by permanent changes in the environment, or (2) by permanent changes in the fundamental organization. In either case the conditions necessary for evolution are provided.

### BIOLOGICAL CLASSIFICATION†

Animals and plants are classified on the basis of their derived organizations. Organisms having common basic structural characters are placed together in one great group, known as a phylum (for example, the phylum Vertebrata consisting of the backboned animals); this phylum is subdivided into secondary groups—classes—each with common characters subordinate to the possession of a vertebral column (*e.g.*, fishes, amphibia, reptiles, birds, mammals). Each of these classes is further divided into orders, and the orders into families; frequently there are sub-orders and sub-families. All this dividing and sub-dividing is on the basis of common structural characters. Finally, we come to genera and then species—minor groups having certain permanent modifications of the structures common to the larger groups. In every case the derived organization must be represented by a certain specific type of fundamental organization of the protoplasm, which, upon development, gives rise to the adult with the special structures characteristic of its derived organization. In all vertebrates, for example, there must be some combination in the fundamental organization that is capable, upon development, of giving rise to the vertebral column, and all vertebrates must have this in common. But in addition to this, amphibia and reptiles must have some combination in their fundamental organization that is lacking in fishes, and

\* See "Heredity and Variation," pages 85-91, in this *Series*.

† See "The Coming and Evolution of Life," Chapter V, in this *Series*.

similarly birds and mammals must have something that is lacking in amphibia and reptiles as well as in fish. In modern genetics it is maintained that a specific characteristic of an adult is represented in the fundamental organization by an hypothetical entity which geneticists term a gene.\* The evidence for this is convincing, as is also the conclusion that the seat of genes is in the nucleus, in which genes are found to be located in the chromosomes—those characteristic bodies which appear at the division period of cells.

On such an hypothesis the chromosomes of a mammal must be exactly as much more complex than the chromosomes of an amphibian as the adult mammal is more complex than the adult amphibian. In other words, the evolution of the fundamental organization must have kept pace with the evolution of the derived organization, not necessarily by an increasing complexity of the genes, but presumably by the increasing complexity of gene associations.

These general considerations in biology are applicable not only to the higher types of animals and plants, but to the smallest living things as well—the protozoa, or unicellular animals, and the protophyta, or unicellular plants. Here also the distinct types of organization permit a classification upon natural (phylogenetic) lines, although the relationships of the various forms are often obscure and frequently conjectural. The idea has been advanced from the evidence revealed by detailed microscopic studies of the cells, that in some protozoa (*e.g.*, *Uroleptus halseyi*) there is only one type of gene per chromosome. The development and differentiation, in such a simple organism, of the fundamental organization into the adult derived organization, although accomplished within the confines of a single cell, are nevertheless unmistakable.

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\* See "Heredity and Variation," pages 52-54 and 65, of this *Series*.

## CHAPTER VI

### THE BORDERLINE BETWEEN PLANTS AND ANIMALS\*

**R**ARELY is the unsightliness of a scum-covered pool due solely to animal life. On the contrary, it is usually brought about by the rich growth of filamentous plants, the blue-green algae or the grass-green algae.<sup>†</sup> These, although beautiful and interesting under the microscope, scarcely come within the limits of the present volume. Living in the water among these plants, however, one always finds a great variety of unicellular animals (protozoa), and unicellular plants, some grass-green in color (e.g., desmids), some yellow (e.g., diatoms), and many chlorophyll-bearing motile forms.

When Leeuwenhoek, in 1675, revealed the world of microscopic forms, he grouped them, plants and animals alike, under the general name *animalcula*.<sup>‡</sup> Later observers, using better microscopes and aided by an increased knowledge of living things, gradually placed the forms which were definitely plant-like in the field of botany and those which were definitely animal-like in that of zoology. Such segregation obviously depends upon our definitions of "plant" and "animal," and is not based upon any clear-cut demarcation in animate forms. As a result it has been impossible to word a definition which will enable us unfailingly and without confusion to place among plants or among animals those unicellular forms which lie on the boundary line between the fields of botany and of zoology. The German biologist, Ernst Heinrich Haeckel, in 1866, sought to avoid the difficulty by creating the term *protista* to designate all unicellular plants and unicellular animals, a term from which we get *protistology* for the science dealing with unicellular life.

\* See "The Plant World," pages 3-9, and "The Animal World," pages 38-40, both in this *Series*.

† See "The Plant World," pages 13-17, in this *Series*.  
‡ *Singular*, *Animalculum*.

## THE TWO CRITERIA

It is a very simple matter to distinguish an ordinary plant from an ordinary animal; the animal moves, sees, hears, tastes, smells, and touches. The plant is stationary, develops wood instead of bone, and has an entirely different method of getting food. The plant has green-coloring matter termed chlorophyll, which, in some obscure way, is instrumental in the nutritional operations of the plant. The two criteria—movement and method of obtaining food—have been the chief features underlying the separation of animals from plants.

It is quite a different matter to determine whether or not a unicellular organism is to be regarded as a plant or as an animal. If it moves, does this fact make it an animal? Obviously not, for there are many organisms which are unquestionably plants (for example, Venus's flytrap and the sensitive plants) which have the power of independent movement, and protoplasmic currents, flow of sap, etc., are characteristic movements of all recognized plants. In the last analysis it is impossible to formulate a definition, or series of definitions, which will enable us clearly to distinguish between all forms of animals and all forms of plants.

## ARBITRARY DECISIONS NECESSARY

Under such conditions it becomes necessary to arbitrate and mutually agree that certain types shall be considered more plant-like than animal-like and certain other types the reverse. This, in effect, has been the case in modern biology, and today it is generally agreed that the presence of chlorophyll, together with cellulose and other carbohydrate compounds formed by the organisms in question, are enough to establish a plant-like nature.

Zoologists, and especially the protozoologists, have been slow to give up the phenomenon of movement as a distinctive animal characteristic, and as a result of this conservatism we find a great many chlorophyll-bearing organisms still retained in our classifications as primitive animals, whereas by tacit agreement their dominant characteristics make them plants. Chlorophyll-bearing forms without motile organs (desmids, diatoms, etc.) have been excluded long since from the animal kingdom, but their chloro-

phyll and the products of chlorophyll action are no different from those of certain other organisms which today are almost universally regarded by zoologists as protozoa. This conservatism is based upon the fact that these chlorophyll-bearing forms of protozoa possess definite motile organs in the form of minute vibrating processes called flagella. Each flagellum grows out as a filament from the substance of a kinetic element\* of granular form

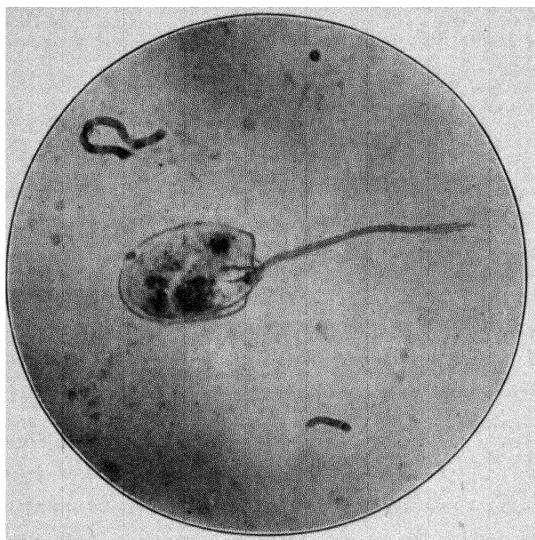


Fig. 19—FLAGELLUM  
STRUCTURE OF  
SCYTONOMAS

From a photomicrograph by  
the author

Magnification, 1000

embedded in the protoplasm. This filament, called the *axial filament*, is covered by a *sheath* when it leaves the body (Fig. 19), and this sheath is a prolongation of the outer membrane (periplast) which covers the entire organism. Many of the chlorophyll-bearing forms which are universally accepted as plants, notably the filamentous algae and the thallophytes† generally, at certain stages of their activity, form actively motile cells which are called *zoospores*. Their motile organs are flagella of exactly the same type as in flagellated protozoa, hence recognized plants have the ability to form motile organs. Furthermore in all of the chlorophyll-bearing protozoa, plant-like resting stages, called the *palmella phase*, are characteristic. At such stages the flagella are lost by resorption and the organisms become embedded in jelly. The length of time in the palmella phase varies—in some groups of the plant flagellates it is the dominant phase (see Fig.

\* See page 72.

† See page 47.

20) and in other groups the motile phase is dominant. On the whole there seems to be little justification for continuing to regard these chlorophyll-bearing flagellates as animals or for retaining them with the protozoa.

### THE MYSTERY OF CHLOROPHYLL

What is this chlorophyll that is so distinctly a plant characteristic? It would not be overstating the matter to say that chlorophyll is the most important type of substance in nature, and that without its activity animal forms would quickly perish. The food of animals if traced back along any line, would finally bring us to chlorophyll-bearing plants and to bacteria. Man, for example, is practically omnivorous, taking his food directly from the plant kingdom in his vegetables and cereals, and from the animal kingdom in his beef, mutton, fish, and fowl. Cattle, sheep, and birds, in turn, obtain their main nourishment from plants. Birds, it is true, eat worms and insects to a great extent, but since worms and insects feed on leaves and vegetables, this food is only one step removed from green matter. Man eats fish, oysters, and other marine food; the larger fish eat smaller ones, and so on until the smallest eat crustacea, larvae, protozoa, and other micro-organisms which finally subsist on microscopic algae and upon bacteria. Back of it all is the mysterious activity of chlorophyll. And yet chlorophyll is not a living substance. It is a product of the activity of certain parts of living plant protoplasm called *chloroplastids*, and these, in sunlight either direct or diffused, have the power to manufacture the highly complicated protein substance, chlorophyll.

It is possible to extract chlorophyll in pure form from some types of plants and to find out its chemical composition. From such analyses it is known that the ordinary green chlorophyll is a combination of green and yellow colored substances and that the proportions of these determine the yellow, brown, or green shade, while the reds are apparently due to the scarcity of nitrogen. If a beam of white light be passed through a prism it is broken up into the seven primary colors of the spectrum. If a similar beam is passed through a column of extracted chlorophyll it is similarly broken up, but some of the rays do not get through, particularly those of the shorter wave lengths (blue

and green regions of the normal spectrum). These are absorbed by the chlorophyll, and the energy which they represent is utilized in some mysterious way not yet understood in the building up of sugars and starches. This phenomenon, *photosynthesis*, whereby chlorophyll with the radiant energy of sunlight, creates organic compounds out of carbon dioxide and water, is the fundamental activity upon which all life is dependent.

There is an almost infinite variety of the microscopic chlorophyll-bearing forms—some of them are permanently quiescent, some continually motile, while others are motile at times and quiescent at other times. Some are always single cells; others are in colony combinations of one kind or another. These are interesting philosophically as pointing the way toward multicellular plants and the possibility of specialization by division of labor.

The power of photosynthesis is the most distinctive feature of plants. Strict construction of this definition of plants, however, would exclude fungi, and many other plant forms which, though colorless, are obviously related to chlorophyll-bearing types. The purpose of biological classification, after all, is to give a common language whereby the subject of observations, experiments, and conclusions may be identified and the results confirmed.

#### CLARITY IN CLASSIFICATION NEEDED

The filamentous algae, desmids, and diatoms, having no motile organs, for decades have been identified as plants and separated as such from the world of microscopic animals, the smallest forms of which are the protozoa. Until quite recently a great many chlorophyll-bearing forms have been retained in systems of classification of protozoa. As the same forms are included in botanical textbooks under an entirely different terminology and classification, a great deal of confusion results. In the interests of clarity it seems wiser for one or the other group of scientists to bow as gracefully as possible to the inevitable and abandon them; and since the questionable forms possess chlorophyll, it is logical that the zoologists should give them up. All of these intermediate forms are characterized not only by the presence of chlorophyll but also by the possession of flagella as motile organs, either permanently or at some stage of their life; hence the name "Flagellata."

## CHAPTER VII

### THE MICROSCOPIC PLANTS—PROTOPHYTA\*

THE group of intermediate flagellates is distributed by botanists among a number of classes. The term protophyta, indicating the same position among plants that the term protozoa does among animals, is rarely used except in a descriptive sense, and then it is interchangeable with the term algae. The organisms included among the algae are by no means limited to the unicellular forms; hence it is impossible in a work on "The Smallest Living Things" to treat all of them. It would be more simple to consider them, as zoologists do, as groups of unicellular organisms without regard to their possible relationships to higher types, than to treat them, as botanists do, as ancestral types of higher forms of plants. The green flagellates in particular are regarded by most botanists as the forerunners of filamentous algae and of thallophytes<sup>†</sup> generally. A captious critic might well question the advisability of assuming that any present day free-living forms are actually ancestral to any present day higher types. With equal authority such forms might be regarded as retrogressive types derived from higher forms of the past, a view that is indeed held by many.

Whichever view we take as to the phylogeny (race history) of these minute forms of life it is expedient to arrange them in some sort of sequence and, as far as possible, in an ascending order of complexity. In transferring the chlorophyll-bearing flagellates to the botanists we abandon a relatively simple classification for one that is far more involved.

In botany the lowest form of the green or chlorophyll-bearing plants is known as the thallophyta, a group distinguished from most of the higher plants by the possession of relatively uncomplicated body structures and by simple reproductive processes.

\* See "The Plant World, pages 13-25, of this Series.

† The thallophytes include the algae, the fungi, and the lichens.

## ZOOLOGICAL CLASSIFICATION OF THE CLASS PHYTOMASTIGODA (PLANT-FLAGELLATES)

*Abridged from Biology of the Protozoa by the Author*

Chlorophyll is present in all, and may be green, yellow, brown, or red in color.

**Order 1. CHRYSOMONADIDA.** Gullet-like region absent; cells not flattened. Chlorophyll yellow.

Sub-order 1. **EUCHRYSMONADINA.** Motile state dominant.

Sub-order 2. **RHIZOCHRYSIDINA.** No flagella; rhizopodia forms.

Sub-order 3. **CHRYSOCAPSINA.** Palmella state dominant.

**Order 2. CRYPTOMONADIDA.** Gullet present; cells usually flattened. Chlorophyll yellow or brown.

Sub-order 1. **EUCRYPTOMONADINA.** Anterior end obliquely truncated. Motile.

Sub-order 2. **PHEOCAPSINA.** Palmella state dominant.

**Order 3. DINOFLAGELLIDA.** One free, one encircling flagellum. Cellulose walls; chlorophyll, if present, usually brown.

Sub-order 1. **DINIFERINA.** Body naked or shell-bearing; latter often complex.

Sub-order 2. **ADININA.** Naked or with bivalve shell; no transverse furrow.

Sub-order 3. **CYSTOFLAGELLINA.** Naked; no groove or furrow; no transverse flagellum.

**Order 4. PHYTOMONADIDA.** Cellulose walls; no gullet; contractile vacuole simple. These are the grass-green flagellates with true starch.

**Order 5. EUGLENIDA.** Green flagellates; paramylum instead of starch; complex contractile vacuole.\*

**Order 6. CHLOROMONADIDA.** Green; complex vacuole, metabolic products oil.

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\* See "The Plant World," page 14, in this Series.

## BOTANICAL CLASSIFICATION OF ALGAE

*From British Fresh-water Algae by West and Fritsch*

- Class 1. **ISOKONTAE.** With grass-green chlorophyll and true starch. The "Phytomonadida" of protozoa are included here together with the desmids (conjugatae) and many filamentous algae.
- Class 2. **HETEROKONTAE.** Also with yellow-green chlorophyll; a small group not separately recognized in classification of protozoa.
- Class 3. **CHYSOPHYCEAE.** Includes types with yellow, orange, or brown chlorophyll. The Chrysomonadida of protozoa are included here together with higher types of filamentous algae.
- Class 4. **BACILLARIALES.** Diatoms, with yellow, green, or brown chlorophyll. No motile organs and not recently included with protozoa.
- Class 5. **CRYPTOPHYCEAE.** With diverse chlorophyll but commonly brown. Included as Cryptomonadida in protozoa. No filamentous forms.
- Class 6. **DINOPHYCEAE.** Chlorophyll when present, yellow, brown or green; with characteristic cell furrows. Included among protozoa as "Dinoflagellida." Except the genus Haplozoon (Fig. 24), no filamentous forms.
- Class 7. **CHLOROMONADALES.** A small group with yellow or green chlorophyll and characteristic structure. Included in protozoa as an Order "Chloromonadida."
- Class 8. **EUGLENINEAE.** With green to red chlorophyll and formation of paramylum instead of starch. In protozoa the Order "Euglenida."
- Class 9. **PHEOPHYCEAE.** Filamentous brown algae. Not included in protozoa.
- Class 10. **RHODOPHYCEAE.** Filamentous red to blue sea-weeds. Never included as protozoa.
- Class 11. **MYXOPHYCEAE.** Blue-green Algae, never included as protozoa.

The two main subdivisions of this group are the fungi and the algae, the latter having chlorophyll by which they are able to create, with the energy of sunlight, the organic compounds of their bodies from carbon dioxide, water, and salts. Fungi, however, have no chlorophyll and lack this power.

According to the classification of West and Fritsch in *British Fresh-Water Algae*, the algae are divided into eleven classes

(see page 49). In the majority of these classes some minute living things are to be found, but only in a few of them are all the types of microscopic size. In practically all these groups motile stages alternate with quiescent phases. The motile phase for the most part is characterized by the presence of from one to four vibratile flagella, and the quiescent phase by the loss of flagella and by the secretion of a jelly within which the individuals are embedded. In this quiescent phase, they may multiply by repeated division until large masses are formed which are sometimes easily visible to the naked eye.

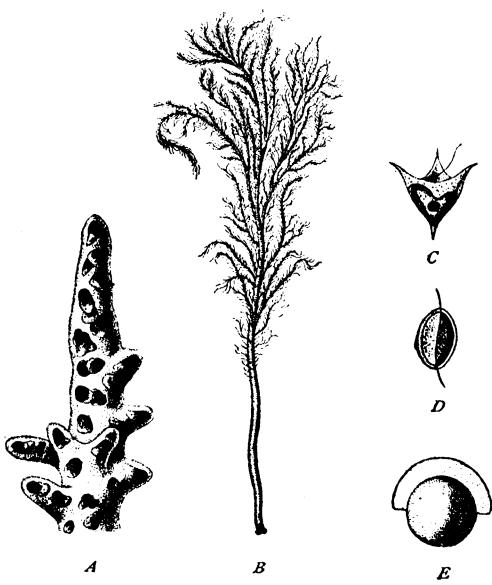


Fig. 20—*HYDRURUS FOETIDUS*

This organism has been called an animal. The bushy growth, B, is made up of branches shown more highly magnified at A, where the yellow chlorophyll-holding cells are embedded in a gelatinous matrix. From time to time swarm cells, C, are formed and swim away. D and E, two views of a cyst formed by the organism.

A, C, D, and E, from Doflein after Klebs; B, from Pascher

Magnifications: A, 150; C, D, and E, 800  
B, 1/3 natural size

This gelatinous, so-called palmella phase does not occur in animal flagellates. In some forms the motile phase is dominant (e.g., Euglenineae), in others the palmella phase from which at times flagellated cells escape for a brief period of motile life, e.g., *Hydrurus* (Fig. 20).

Colonial forms likewise occur in nearly all groups. Colony formation may be accompanied and brought about by the secre-

tion of a jelly recalling the palmella phase. Such groups, known as *spheroidal colonies*, may be provided with flagella, and their movement is active, *e.g.*, *Gonium pectorale* (Fig. 21) and *Uro-*

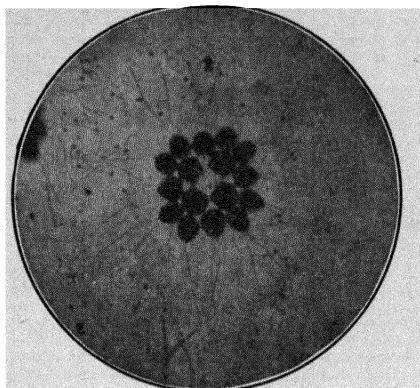


Fig. 21—*GONIUM PECTORALE*

This sixteen-celled colony of one of the green plant flagellates is not often caught with all of its flagella showing

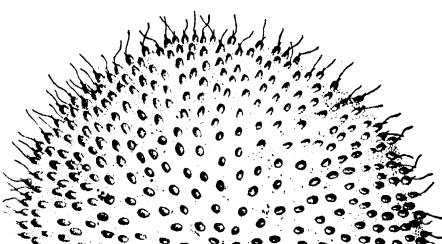
*From a photomicrograph by the author*  
*Magnification, 290*

*glenopsis Americana* (Fig. 22), or they may be quiescent (*e.g.*, *Coelosphaerium*, *Microcystis*, and *Aphanocapsa*). In other cases, as in some types of bacteria, colony formation is due to the adhesion of cells after division, forming so-called "catenoid

Fig. 22—A. YELLOW-COLORED  
PLANT FLAGELLATE, *URO-*  
*GLENOPSIS AMERICANA*

A delicate, gelatinous colony of minute yellow zoospores which are embedded in a gelatinous matrix. Each zoospore contains one or more oil globules which are liberated when a colony is broken up in water pipes and cause a fishy odor in the water

*From a drawing by the author*  
*Magnification, 250*



(chain-like) colonies," in which the cells adhere side by side or end to end (Fig. 24), or "arboroid (tree-like) colonies" in which the cells are attached alternately by stalks or tests\*, (Fig. 23). Division in two planes gives rise to thallus or plate-like colonies (Fig. 24). Attachment of individuals in a linear series gives rise to filamentous forms, a characteristic of the brown, red, and blue-green algae.

\* Test, the external, firm covering of a cell; it is not a living part of the cell, and varies in consistency in different forms from rigid to soft and gelatinous.

In this brief review of the smallest living things belonging to the vegetable kingdom it is necessary to choose somewhat arbitrarily from the enormous mass of material found in text-

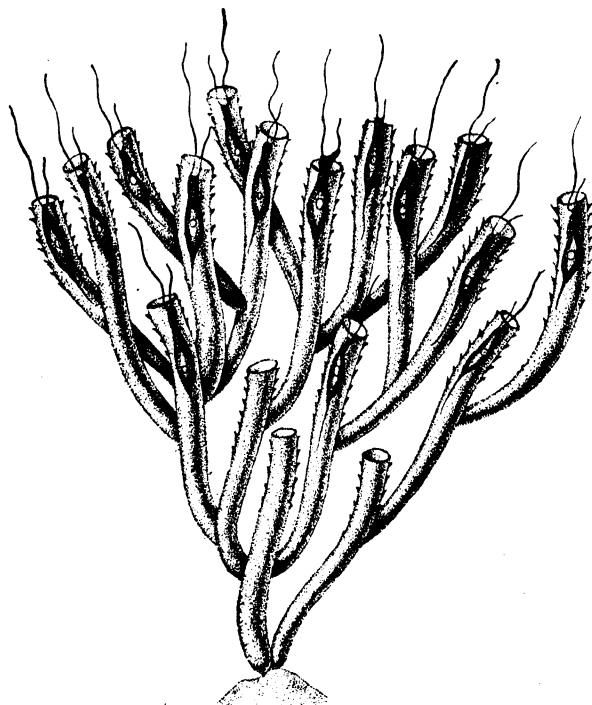


Fig. 23—A TYPE OF ARBOROID COLONY OF THE YELLOW FLAGELLATE, HYALOBRYON DEFORMANS

Here the daughter individuals which are formed by division attach themselves to the outside of the parent cup and there secrete their own cups

*From a drawing by M. Valentine after Lauterborn  
Magnification, 800*

books and monographs.\* I shall select only those forms which strike the eye of an observer using a microscope. For the most part these fall in the classes Isokontae, Chrysophyceae, Bacillariales, Cryptophyceae, Dinophyceae, and Euglenineae of the eleven classes listed on page 49.

\* For complete treatment, consult Engler and Prandtl, *Natürlichen Pflanzenfamilien*, or West and Fritsch, *British Fresh-water Algae*, and special monographs on desmids, diatoms, dinoflagellates, and the like.

## CLASS ISOKONTAE

All the Isokontae\* are plant-like forms in which the chlorophyll is green and made up of two green and two yellow pigments in approximately the same proportions as in higher plants. True starch is found as a characteristic product of nutrition, and cellulose is a main constituent of the cell wall. The forms included here are widely distributed in fresh water ponds and pools but rarely occur in salt water. Theoretically, they are interesting in that, more than any other group of algae, they are regarded as standing in the direct line of evolution of the higher plants; in other words, they are considered to be related to ancestral forms of the present day higher groups.

The simplest and smallest of these forms are freely motile (Fig. 25) and the palmella phase is either absent or temporary, but colony formation is very common and the colonies are motile. In some of the larger and related types

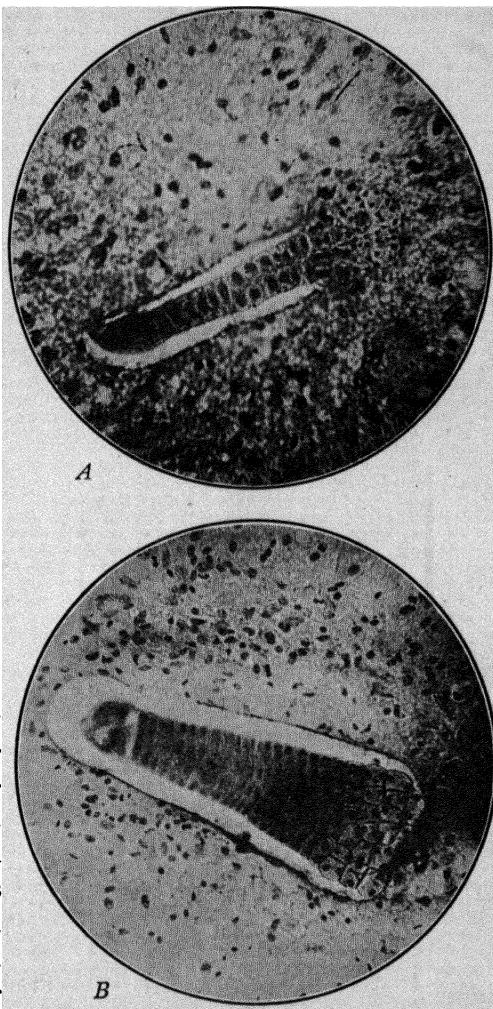


Fig. 24—*HAPLOZOON CLYΜENELLAE*

Parasites of the digestive tract of a marine worm. They are highly modified dinoflagellates, one type, *A*, recalling the structure of filamentous algae, the other, *B*, the plate-forming habit of thallophytes

From photomicrographs by the author  
Magnification, 170

\* The term Isokontae refers to the equal-size flagella or motile organs on the gametes.

the palmella condition is the dominant form and non-motile colonies are very characteristic, although the individual cells readily revert to the motile phase. The smaller motile forms are included for the most part in the group represented by the globular colony of *volvox* (Fig. 26), but equally small non-

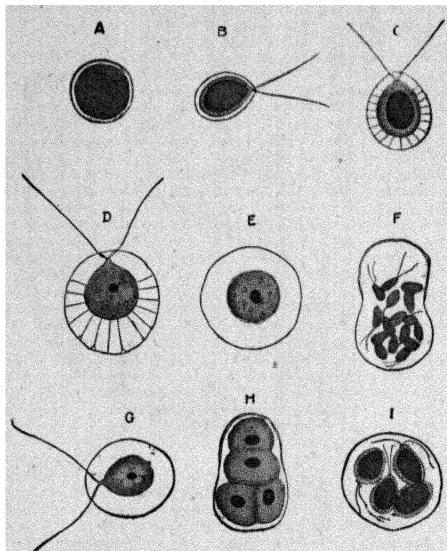


Fig. 25—A PLANT FLAGELLATE,  
*SPHAERELLA LACISTRIS*

It may be motile or quiescent, red, green, or colorless. *A*, a red-colored resting form; *B*, a red-colored, biflagellated motile form; *C, D*, green-colored motile forms; *E, F*, parent cell and flagellated zoospores from it; *G, H, I*, a green cell goes into palmella stage and forms four red motile cells

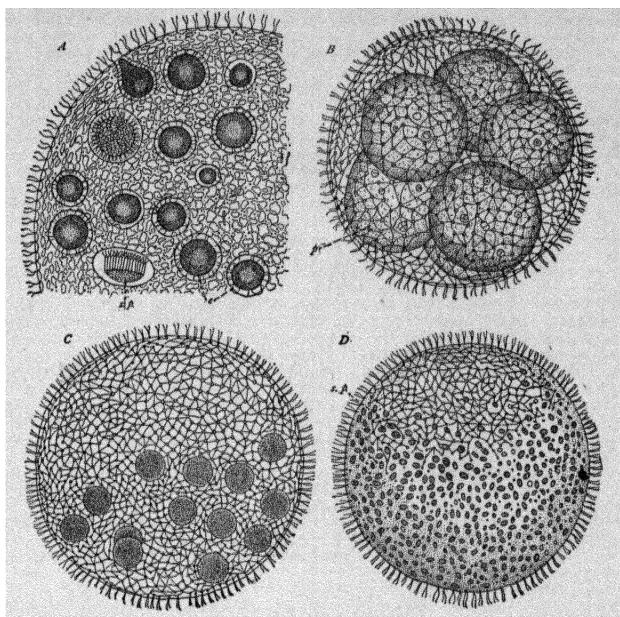
After T. E. Hasen  
Magnification, 600

motile forms, either alone or in the palmella phase, may develop flagella and become motile for a short period, *e.g.*, the chlorococcales. Still another group of small forms—the desmids—are permanently quiescent and do not have a palmella phase. In other groups of this class, the organisms are filamentous and are usually found in aggregates which are visible to the eye. In the present volume, therefore, we shall limit treatment of the isokontae to these three non-filamentous forms, in all of which grass-green chlorophyll is characteristic.

#### *The Volvox Group*

In the first of these, the *volvox* group, the central unicellular type is well represented by small unicellular forms of the genus *Sphaerella* (Fig. 25). Two equal flagella found at the anterior end draw the cell briskly through the water while at the same time it is rotating on its long axis. A definite membrane covering the cell gives rigidity to the body, so that change of shape

is impossible. Reproduction occurs by division after the flagella have been withdrawn. Unlike animal flagellates the volvox group have a sexual phase in which gametes of equal size (isogametes) are produced. These gametes are formed by the re-



*A*, a quarter of a colony of *Volvox globator*. This species is hermaphrodite,\* and eggs (*e*) and sperm cells (*sp*) are formed from cells which wander into the colony from the periphery (*p*). *B*, parthenogonidia-forming colonies of *Volvox aureus*. *C*, female colony with unfertilized egg-cells. *D*, male colony with many bundles of spermatozoids

*From Oltmanns*  
*Magnifications: A, 60; B, C, and D, 40*

peated divisions of an ordinary cell, and in consequence are considerably smaller than the usual individuals resulting from ordinary division.<sup>†</sup>

Colony formation is highly developed in this group, the colo-

\* Hermaphrodite, an individual having both male and female reproductive organs.

† Parthenogonium (*plural*, parthenogonidia), an individual which can produce a colony by one of the asexual methods—cell-division, spore-formation, budding, etc., but does not include the self-fertilization of hermaphroditic organisms.

† In connection with this group it is interesting to note that the German botanist, Adolf Pascher, was able (in 1916) to demonstrate a typical Mendelian inheritance in these forms by crossing two different species of the related genus *Chlamydomonas*. This is the only case in which a typical Mendelian inheritance has been demonstrated for an unicellular organism.

nies, in the majority of species, being made up of a definite number of cells grouped in definite arrangements. Thus there are

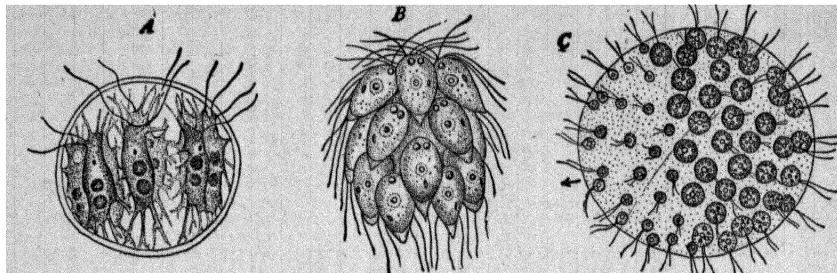


Fig. 27—COLONY FORMS OF GREEN FLAGELLATES

**A**, a colony of eight cells, *Stephanosphaera pluvialis*, after Kühn; **B**, a colony of 16 cells, *Spondyliomorium quarternarium*, after Stein; **C**, a colony with two kinds of cells (the small cells are vegetative cells, the large ones are germinal), *Pleodorina californica*, after Chatton

*Magnifications: A and B, 500; C, 120*

eight cells in some, sixteen in others, thirty-two in others, etc. (Fig. 27). In *Pleodorina* from Illinois (*Pleodorina illinoensis*) there are thirty-two cells in the colony, but in the form from California (*Pleodorina californensis*), however, there are many more than this number, those in one hemisphere of the colony being much larger than those in the other (Fig. 27c). Each individual of a sixteen-cell colony of *Gonium pectorale* may reproduce the entire colony by continued division (Fig. 28), but in *Pleodorina* certain cells only have this power, the remainder disintegrating. In *Volvox* colonies, consisting of many hundreds of cells, certain cells (parthenogonidia) withdraw from the periphery and produce other colonies within the jelly sphere (Fig. 26).

Sexual phases reach a high development in these types. In the majority of forms the male and female gametes are similar in shape and size. In *Eudorina* practically all of the thirty-two cells may form gametes, termed in this case anisogametes (unequal gametes), since some of the cells form larger gametes which serve as eggs, while others form smaller gametes which serve as sperms. In *Volvox* only a limited number of the cells form gametes, a fact which, together with the presence of vegetative cells and of parthenogonidia, or colonies that reproduce asexually, indicates a relatively high division of labor (see page 10).

In all cases the fusion of the gametes in this group is complete, resulting in a zygote\* which may become a zygospore by developing a resistant membrane which is frequently ornamented with short spiny processes.

The motile phase is dominant in all of the above forms, the jelly-invested or palmella stages being transient or absent altogether. In other related groups, however, the motile stages are of short duration and the palmella stage is the dominant form. The type genus here is *Palmella*, in which form the individuals are minute green cells surrounded by gelatinous coatings which fuse into a homogeneous matrix—hence the term “palmella phase” as applied to other organisms. In some cases the cells fuse in the form of long gelatinous rods; in other cases in groups of four cells. Zoospores, or flagellated individuals, and isogamous gametes are known in practically all types.

#### *Group Chlorococcales*

In many of the simpler and non-colony-forming types of the *Volvox* group, the flagella are withdrawn during the process of cell division. If such a transient, motionless stage should be

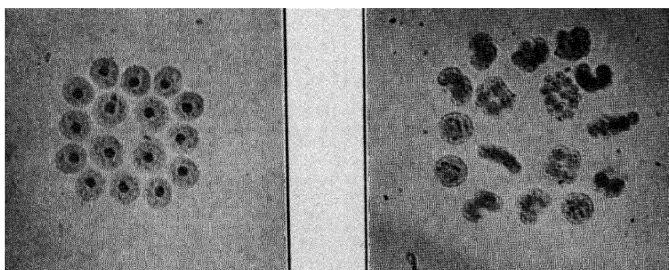


Fig. 28—*GONIUM PECTORALE*

A common form of colonial fresh-water plant flagellate. *Left*, an ordinary vegetative colony; *right*, the same in reproduction; each individual of the ordinary colony forms a sixteen-cell colony

*From photomicrographs by the author  
Magnification, 400*

extended for long periods, there would result a type with the main characteristics of the group *Chlorococcales*. In this type, the solitary individual loses its flagella and grows to a certain size,

\* Zygote, a fertilized cell.

after which it divides into a definite number of cells, each of which acquires two flagella and escapes as a swarmer (free-swimming cell). This soon loses its flagella and repeats the process. Many of these simpler forms acquire a saprophytic mode of life, one outcome of which is the formation of associations with other organisms in symbiosis.\* Thus the "green"

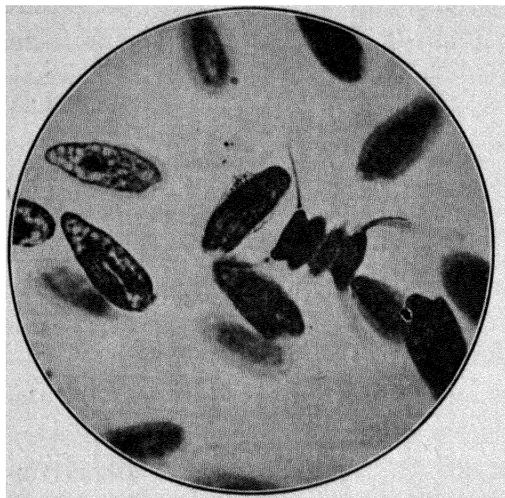


Fig. 29—SCENEDESMUS

The creature with four horns is a very common cause of the green coating on water pitchers and in glasses which have stood in the light for some days. The other creatures in the field are perhaps the most common type of flagellates in stagnant water,  
**CHILOMONAS PARAMECIUM**

*From a photomicrograph by the author  
 Magnification, 600*

cells of the protozoan *Paramecium bursaria* and of fresh-water sponges are really the cells of the protophyte *Chlorella* which live and multiply in these novel environments, to the mutual benefit of both members of the association.

Many of these forms, just as in the *Volvox* group, are associated in colonies of different kinds. One of the most common of these is the four-cell colony, *Scenedesmus* (Fig. 29); others contain from two to thirty-two cells, and still others many cells. These are all very common in drinking water and account for the green color in pitchers, vases, etc., containing standing water.

### *The Desmid Group*

The desmids, which are exclusively fresh-water plants, are widely represented in the ordinary pools and lakes of all regions. In 1902 there were known about 2000 species distributed in thirty-one genera, and many more have since been added. They vary in size from  $8\mu$  to  $1200\mu$  in length and are peculiar in being

\* See the Glossary of this book, page 123.

bilaterally symmetrical.\* In the majority of species of this group a central constriction called "the isthmus" divides the organism into two equal parts, each half being termed a semicell. In the genus *Closterium* (Fig. 30) a constriction of the cell body is present but the cell wall remains intact, while in a few genera, *e.g.*, many species of *Penium*, there is no evidence whatsoever of a constriction. The genus *Cosmarium* is the largest in number of species, many of them having exquisitely ornamented cell walls showing symmetrical patterns in the arrangement of granules, and of the papillae or knobs of various forms and sizes (Fig. 31—*Xanthidium*). The greatest diversity of forms in any one genus is found in *Staurastrum*, common in reservoirs and lakes, while the most spectacular forms in respect to minute symmetry are certain species of *Micrasterias*. The outer wall in all genera of this group consists of two valves, one for each semicell. One of these fits into the other like a pill-box into its cover.

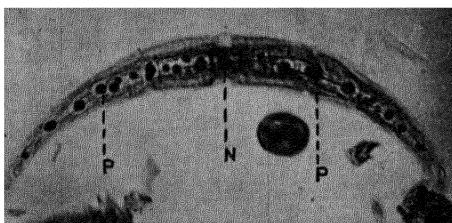


Fig. 30—CLOSTERIUM

One of the largest of the desmids. The nucleus, *N*, indicates the meeting place of the two valves. A number of pyrenoids, *P*, sometimes called starch-forming nuclei, are arranged symmetrically

From a photomicrograph by the author

Magnification, 160

Courtesy of the American Museum of Natural History

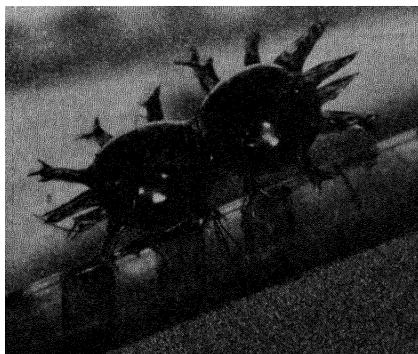


Fig. 31—A MICROSCOPIC WATER PLANT

*Xanthidium armatum* is composed of two connected cells armed with spines, which enable it to cling to plant stems

Magnification, 480

plants occurs by a peculiar process of budding, a type of division which in the simpler desmids consists in the formation

\* Bilateral symmetry is the condition of having the right and left sides of the body similar; the majority of metazoa exhibit bilateral symmetry in their external forms.

Reproduction of these

of a band of protoplasm in the isthmus formed by the constriction. With its formation the two semicells are pushed apart. A division plane passes through the center of the band of protoplasm and two daughter desmids result, each with one old semicell and a new bud. The latter soon grows to the size of the old semicell and the division is completed. In some types the daughter cells remain united, thus forming chains of cells or filaments, as in the genus *Desmidium*.

In many desmids no sexual processes are known, but in some forms a peculiar type of conjugation\* occurs which resembles the fertilization processes of many kinds of filamentous algae and justifies the inclusion of desmids with the latter in the group *Conjugatae*. In this process two individuals come together and are enveloped in a common gelatinous matrix. A tube-like outgrowth emerges from the isthmus of each desmid and in these tubes the protoplasmic bodies meet and fuse to form the fertilized cell, or zygote. The zygotes develop heavy coatings for protection, and their germination occurs after a prolonged resting period.

#### CLASS CHRYSOPHYCEAE

In botanical textbooks the order "Chrysomonadida" of protozoa is termed the Chrysophyceae. Here the green chlorophyll, which in normal plants is composed of four pigments (two yellow, two green), becomes yellow by the preponderance of one of the yellow pigments (phytochrysin). Products of nutrition are stored up in each cell in the form of oil and of highly refrangible, colorless rounded bodies composed of a substance known as leucosin, the chemical composition of which is still uncertain. These leucosin granules are characteristic of the class. The oils frequently cause disagreeable odors and taste in drinking waters—*Uroglonopsis* (Fig. 22, page 51) and *Synura* (Fig. 32) being particularly noteworthy in this respect.

In the cells of this group the flagella, one or two in number, are delicate whip-like structures. They are inserted at the forward end (the apex) of the cell, and are very difficult to see without the use of reagents. The cell bodies are comparatively simple in structure and are usually covered by a delicate, color-

\* See "The Plant World," pages 14 and 25, in this Series.

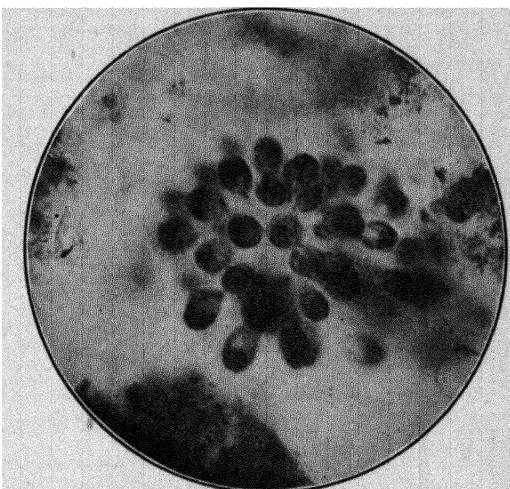
less membrane, but in many cases this membrane is conspicuous because of markings, ridges, flanges, etc., or is thickened by the deposit of silicious or calcareous plates. Cups (or houses) and stalks, usually of cellulose, secreted by the organisms themselves, are not uncommon.

Sexual processes are entirely unknown in this class, reproduction occurring by the longitudinal division of the cells. The daughter cells in many cases become independent after division, but there is a tendency in the group for the cells to remain connected in one way or another so as to form colonies. Sometimes

Fig. 32—*SYNURA UVELLA*

A colony of yellow-colored flagellated cells which are attached by their inner ends. Each cell has two flagella and two chromatophores. *Synura* is a frequent cause of a "cucumber-like" odor in drinking waters

From a photomicrograph by the author  
Magnification, 600



they remain associated side by side, thus forming catenoid colonies; or they remain attached by stalks or cups and form branching or arboroid colonies; or they may remain embedded in a gelatinous matrix, thus forming spheroidal colonies.

Many of the Chrysophyceae have the ability to discard their flagella, round out, secrete a jelly and pass into a palmella stage, during which reproduction takes place. In some types this is only temporary, the active phase being quickly resumed. In others, however, this palmella stage is the dominant phase (e.g., *Hydrurus*, Fig. 20, page 50), and the resemblance to higher types of algae is obvious.

The power of encystment is also characteristic. In this process a monad withdraws its flagella and secretes an impervious membrane, within which it is protected against adverse en-

vironmental conditions. In the Chrysophyceae the cyst wall, made of silica, has a single opening or pore, and is first laid down within the organism, a layer of protoplasm covering the cyst on the outside. This outer protoplasm, after variously sculpturing the outside of the cyst wall, re-enters through the pore, which is then securely plugged from within with a glass-like stopper cemented with silica. Within this glass bottle the monad may reproduce by division or lie dormant for long periods.

### CLASS BACILLARIALES (DIATOMS)

In every collection of material for microscopical study secured from a pond or a ditch, bright yellow-colored organisms of definite shapes will always be found. These are the diatoms, which constitute another group of algae with about the same size variations as the desmids. They are far more numerous than desmids, however.\* They are also much more cosmopolitan than desmids, occurring in seas, especially in the colder waters, as well as in fresh water. In the sea they form a great proportion of the plankton<sup>†</sup> and serve as food for animals of all kinds and sizes. In fresh water they occur in sediment, or attached to filamentous algae, or float as suspended organisms in the clear water. Their yellow color is due to the presence of chlorophyll in which the green color is masked by the deposit of a special yellow pigment known as diatomin. This may be removed by the use of dilute alcohol and the true green color of chlorophyll is then exposed.

In structure they are not unlike the desmids, each individual consisting of two valves called frustules—fitted together like a pill-box and its cover. It is generally agreed, however, that this resemblance is due to parallel evolution rather than to any genetic relationship of the two groups. One valve, corresponding to the cover of a box, is measurably larger than the other. Both valves are richly impregnated with silica which makes them prac-

\* Karsten (Engler and Prandtl, 1928) enumerates no less than 189 genera, and Fritsch (*Encyclopedie Britannica*, 1928, "Bacillariales") enumerates upward of 1500 species and this number does not include marine forms.

† Plankton, the passively floating or weakly swimming animal and plant life of a body of water; it is chiefly minute organisms, diatoms and blue-green algae among plants and protozoa, various eggs and larvae and the slow-moving jellyfishes. Fishes and other large actively swimming animals are not included.

tically imperishable. In consequence there is a constant settling on lake or sea floor of shells coming from dead diatoms, from valves discarded at reproduction and spore formation, and from shells remaining after digestion and defecation by marine animals. Masses of such accumulations\* raised above sea-level by upheavals from time to time upon solidification have formed masses of "diatomaceous" earth in which the shapes and markings of the individual diatoms are still intact. These earths sometimes cover an area of many miles, and at some places, as near Richmond, Virginia, the beds are forty feet in depth, while in some of the western States large deposits up to 300 feet in thickness have been discovered. These earths are usually gray or white in color, and being quite easily pulverized they have several economic uses, as for example in so-called tripoli polishing powders (Kieselguhr), dentifrices, non-conducting materials, or as absorbents for nitro-glycerine in the manufacture of dynamite.

Each diatom may be observed from two aspects: 1, from that in which one or the other valve is seen (valve view); 2, from that in which the girdle is exposed to view (girdle view). In the majority of diatoms (the Pennatae) the face of each valve is marked by a central line (raphe) which is interrupted in the middle by a nodule, and terminates in a nodule at each end. Delicate markings, composed of minute pores in the shell, are arranged symmetrically on the two sides of each raphe and find useful application as test objects for microscope lenses. This type of diatom has the power of independent movement which, according to the observations of competent authorities, is due to the secretion of a gelatinous material which comes out through these delicate pores and through the raphe. In another and a smaller group (the Centricae), most of which are marine and motionless, the valves are circular and without central lines, the valve markings being arranged radially with respect to a central point.

The chromatophores<sup>†</sup> vary from one to many in number and are also highly variable in form. Among the products of assimilation stored up in the cell are variously colored droplets of oil. These are probably the source of occasional unpleasant odors and tastes in drinking waters. The genus *Asterionella*, for ex-

\* See "The Plant World," pages 16-17, in this Series.

† See the Glossary of this book, page 120.

ample, may grow luxuriantly in certain pure lakes and reservoirs, particularly in spring after the reservoirs have been frozen during the winter. At such times the water has a not unpleasant geranium-like odor which is unquestionably due to *Asterionella* and probably to their contained oil drops.\*

The method of reproduction of diatoms recalls that of the budding division of the desmids. Multiplication takes place usually at night and is preceded by a slight increase in the volume of the cell. The color-bearing chromatophores and the nucleus divide, their division being followed by that of the protoplasmic mass in a plane parallel with the valve faces. New, very delicate, siliceous valves are secreted about the daughter cells and within the older valves, but with the development of the connecting bands the old valves are forced apart. Being contained within the older frustule it would appear that the daughter diatoms must be smaller than the parent cell from which they came. In some cases this appears to be the case. In other cases, however, the daughter valves are only slightly silicified and may undergo an increase in size before strong silicification sets in.

A process resembling that of zygote formation in desmids is also common among diatoms. This "auxospore" formation varies widely in different types. In some (*e.g.*, *Navicula amphibena*), the protoplasm of one of the smaller cells, while still embedded in jelly, swells up, escapes from its frustule as an auxospore and then grows to the full size of the species. In others, the protoplasm divides into two cells, each of which forms an auxospore, *e.g.*, *Synedra affinis*, or the two cells thus formed reunite to produce one auxospore, *e.g.*, *Achnanthes subsessilis*, and in still others the protoplasms of two associated individuals leave their frustules, fuse, and form a single auxospore, *e.g.*, *Surirella*. In several species each of the two associated frustules divides into two cells, these fuse with the two from the other individual, and two auxospores result. There are wide variations between parthenogenesis and fertilization processes in this group.

Still another method of multiplication occurs in some forms. This consists in the multiple division of the cell whereby a number of spores are formed. In certain marine types these spores

\* Consult G. N. Calkins, *A Study of Odors Observed in the Drinking Waters of Massachusetts*, Reports, Massachusetts State Board of Health, 1892.

are provided with flagella and there is a tendency to regard them as gametes, but conclusive evidence of this is lacking.\*

### CLASS CRYPTOPHYCEAE

The order Cryptomonadida of protozoa becomes the Class Cryptophyceae of the botanists, and is made up for the most part of marine forms varying in size from  $30\mu$  to  $80\mu$ . These differ from Chrysomonads in having a constant body form, in being laterally compressed, and in having a more or less distinct furrow or gullet. Many forms are colorless, but in the group as a whole, chromatophores of yellow, brown, blue, blue-green, and green color may be present, the color being dependent upon the proportions of the four pigments (see page 45). The colorless forms are saprophytic, *i.e.*, they obtain their nutriment by absorption of dissolved proteins and carbohydrates which are present in the surrounding medium. Such forms are common, therefore, in stagnant pools, and one type, *Chilomonas*, is easily found (Fig. 29, page 58). As with other groups, so here, we find forms in which the motile stage is dominant, *e.g.*, *Cryptomonas*, *Chilomonas*, *Nephroelmis*, and other forms in which the palmella state dominates. Motionless unicellular individuals are also well known, and these, like *Chlorella* among the Chlorococcales, may become symbiotic with animal cells. The best known examples of this association (symbiosis<sup>†</sup>) are the "yellow cells" (*Zoöxanthelleae*) of Radiolaria and Foraminifera (see pages 76-78).

### CLASS DINOPHYCEAE (PERIDINIALES)

By far the most interesting of the plant flagellates in respect to variety and power of adaptation are the Peridiniales of the botanists or the Dinoflagellida of the zoologists. Some types are sure to be found in stagnant pools or in reservoirs, *e.g.*, *Ceratium tripos* or *Ceratium hirundinella* (Fig. 33), but the great majority of the group are marine and *Noctiluca miliaris* in large part is responsible for phosphorescence in the sea. Although

\* For a general description of diatoms, consult West and Fritsch, *British Fresh-Water Algae*, 1927 edition; or Engler, *Naturlichen Pflanzenfamilien*, Vol. II, 1928. For the determination of species, consult W. Smith, *A Synopsis of the British Diatomacea* (1853-56), or V. Schonfeldt in Pascher's *Süsswasserflora Deutschland's*, etc., Jena, 1913. For marine forms, consult Karsten, "Bacillario-phyta" in Engler's *Naturlichen Pflanzenfamilien* cited above.

† See the Glossary of this book, page 123.

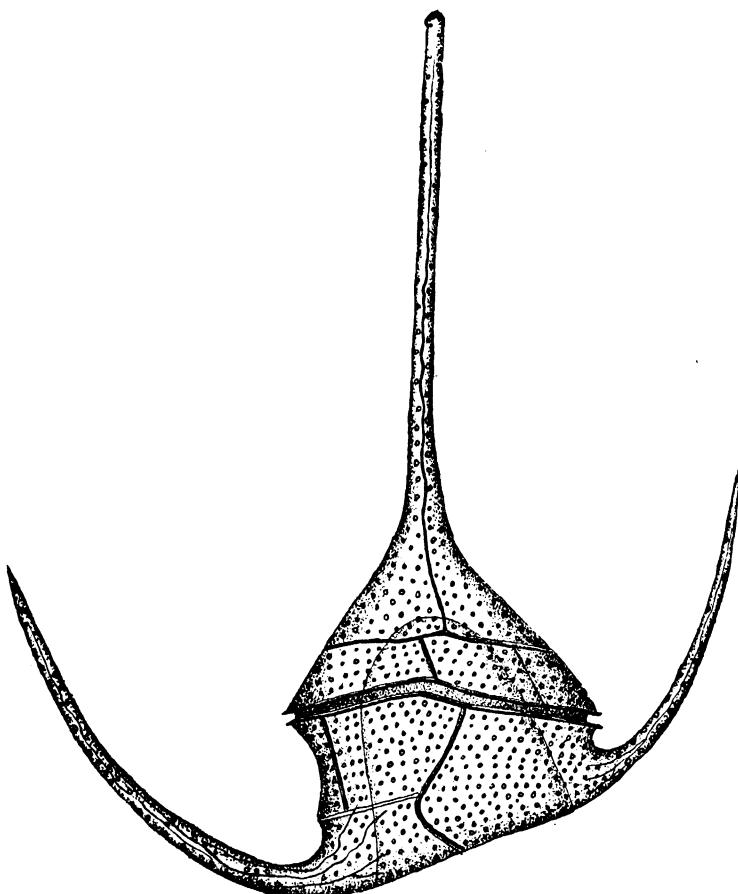


Fig. 33—AN ABORAL\* VIEW OF THE SHELL OF A COMMON FRESH-WATER DINOPHYCEAE, CERATIUM TRIPPOS

Sutures show the limits of the shell-forming plates; the groove (annulus) is formed by a single plate. Certain plates are drawn out into horns, two on the hypotheca (lower half), and two (fused) on the epitheca (upper half)

*After Stein  
Magnification, 400*

many of them are naked, most of them are covered by a definite membrane or test, mostly of cellulose but often impregnated with inorganic, sometimes calcareous, deposits. The test may be simple and made up apparently of one piece, or it may have two valves, or it may be composed of plates of definite form and arrangement. Such plates are often drawn out into typical horns

\* Aboral (ăb-ō'răl), opposite to or away from the mouth.

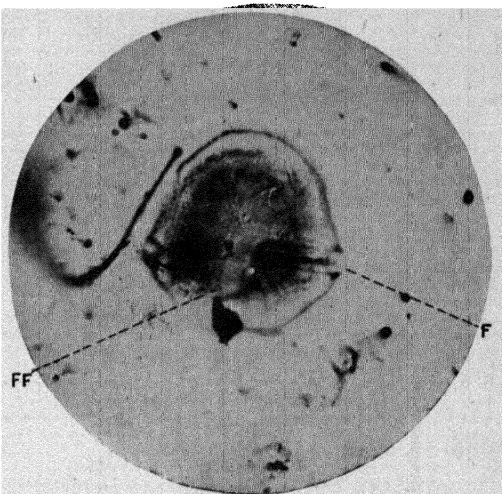
and processes and frequently give rise to fantastic shapes (Fig. 33).

These organisms are unique in having a characteristic groove (annulus), which usually encircles the cell. It is present in both naked and shelled forms and provides a furrow in which lies one of the two flagella (Fig. 34). This furrow is sometimes at the extreme end of the cell but in others it apparently disappears entirely, leaving the encircling flagellum free in the water (Ex-

Fig. 34—*GONYAULAX APICULATA*

A common fresh-water dinophyceae with characteristic furrow running about the body. There are two flagella: one, *F*, occupies the transverse furrow, the other, *FF*, extends out into the water

From a photomicrograph by the author  
Magnification, 600



uviaella). The second flagellum is more thread-like and vibrates freely in water.

Chromatophores of green, yellow, or brown color, and varying in number from two large bodies to many small disk-shaped structures, may be present, but are absent here and there in representatives of all families of the group and in all of the deep-sea forms. The type of food-getting varies accordingly, from a strictly plant type (holophytic) to a strictly animal type (holozoic), although there are some remarkable adaptations to a parasitic mode of life (Blastodiniaceae), a most extreme case being that of *Haplozoon* (Fig. 24, page 53), a parasite found in the intestines of marine worms. In this form there is a return to the filamentous type of algae.

Reproduction is effected by division of the cells into two, usually along the longitudinal axis. In *Ceratium hirundinella* the complex shell splits between definite plates, the protoplasm di-

vides, and the daughter cells then require some time before they assume the characteristics of the species.

### CLASS EUGLENINEAE

This group, the Euglenida of protozoa, is another class of plant flagellates, representative species being found everywhere in

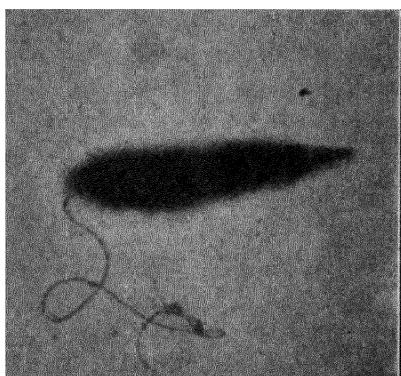


Fig. 35—A COMMON FLAGELLATE,  
*EUGLENA GRACILIS*

Photographed to show the length of the flagellum

From a photomicrograph by the author  
Magnification, 950

stagnant waters. They are quite complex structurally, but are rarely colonial, the independent cells moving about by means of one or sometimes two flagella. Green chlorophyll is present in chromatophores, varying in number from one to many. Instead of starch, prominent products of assimilation appear as solid, frequently large, granules of paramylum\* of diverse shapes (rods, rings, disks, etc.). The individual cells vary in size from  $15\mu$  to  $150\mu$  and are usually elongate and cylindrical with tapering to pointed posterior ends. *Euglena* (Fig. 35), the commonest genus, and many other related genera, have red eye-spots;† *Phacus* is flattened and has one central paramylum granule; *Trachelomonas* is enclosed in a shell which is often ornamented with spines, or spiral ridges. *Palmella* states are common; the individual discards its flagellum, becomes rounded, and secretes a gelatinous envelope in which it divides. Many colorless types are known, of which one of the commonest, *Peranema*, moves slowly along the substratum‡ by means of a tiny whip lash (Fig. 36).

\* Paramylum is a carbohydrate, analogous to starch, but it does not stain with iodine.

† See the Glossary of this book, page 121.

‡ Substratum, the substance or base upon which a plant grows.

Reproduction is by longitudinal division, sometimes occurring in the free-swimming state. Fertilization processes have been re-

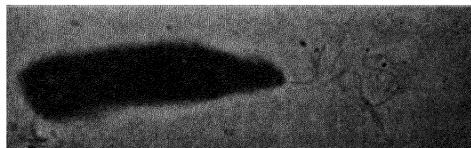


Fig. 36—*PERANEMA TRICHOPHORA*

A flagellate which creeps along slowly with its flagellum extending straight ahead, the tip only in vibration

*From a photomicrograph by the author*  
*Magnification, 800*

ported for *Euglena sanguinea*, but the observations thus far have not been confirmed.

## CHAPTER VIII

# THE MICROSCOPIC ANIMALS—PROTOZOA

### TYPES OF PROTOZOA

**I**N SAMPLES of water taken from a pool or pond and examined under the microscope there are quantities of actively moving forms of life which quickly catch the eye. Many of these, indeed nearly all of the brightly colored ones, are the unicellular plants which were considered in the preceding chapter. Others are protozoa, one-celled animals, and these represent forms related to what was probably the vanguard of the long procession of animal types culminating in man. Some of them have no motile organs, but creep about on the bottom, and afford a fascinating picture of protoplasmic moving, *e.g.*, *Ameba*. Others move with microscopic rapidity, turning on their axes or progressing in a zigzag course through the water. The motile organs of these forms, called flagella, are, owing to their fineness and to their active movements, difficult to see, even under very powerful lenses, but are revealed by adding a drop of iodine to the preparation. They extend from the forward (anterior) end of the cell into the water as one, two, or more exquisitely fine, hair-like processes. In life they undulate from base to tip and at the same time describe a cone the apex of which is the point of insertion in the body. In still another type of protozoa the movement is more regular, but is also a combination of rotation and forward progression. In these the surface of the cell may be seen to be covered with a coating of beating cilia or with a conspicuous zone of cilia at the anterior end. These, like flagella, are readily demonstrated by the use of iodine.

These forms, ordinarily found in stagnant water, represent three of the four great groups or types of organization of the protozoa — *Mastigophora* (whip-bearing), *Sarcodina* (from *sarcode*, an early term for protoplasm), and *Infusoria* (living in infusions). The fourth type, *Sporozoa*, are not found in water.

for these are obligatory parasites\* living in the fluids and cavities of all kinds of animals. We will discuss the first three types in this chapter, but the Sporozoa will be considered in a later chapter (see page 105). Altogether there are upward of 15,000 known species of protozoa, varying in size from half a micron to half an inch (Porospora, a parasite of the Norwegian lobster) for single individuals with one nucleus, and up to  $2\frac{1}{2}$  to 3 inches for individuals with many nuclei (Nummulites).

### ANIMAL FLAGELLATES (MASTIGOPHORA)

#### *Characteristics*

The typical form of an animal flagellate is ellipsoidal, or elongated in the direction of movement (Fig. 37). Its protoplasm is usually somewhat denser on the outside, thus forming an indefinite cortical zone rather than a definite membrane.

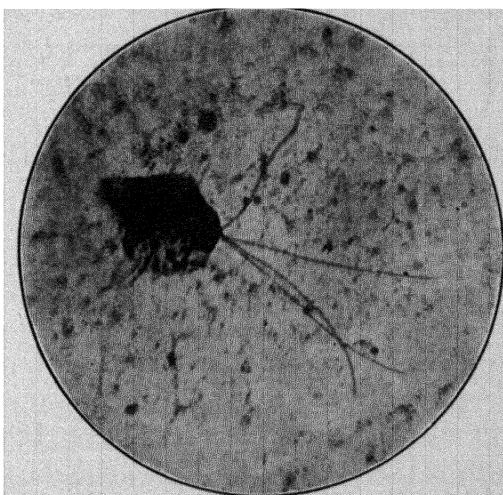


Fig. 37—COLLODICTYON

A highly voracious animal flagellate with four flagella

From a photomicrograph by the author  
Magnification, 720

Closely attached to it, however, on the outside is a delicate, usually invisible, lifeless covering known as the periplast or pellicle. This, indeed, is present in all protozoa. In a strict sense, there are no naked forms of protozoa.

The smallest and the simplest of the flagellates are the most important in relation to man. Here the derived organization (see page 38), typical of the Mastigophora, is seen in its sim-

\* Obligatory parasites cannot live under any but parasitic conditions.

plete expression and may be characterized briefly as an ellipsoidal protoplasmic body bearing one or more flagella at the anterior end. In their finer structure the flagella are similar throughout the group and are always anchored deeply within the protoplasm, thus differing from the so-called flagella of bacteria and spirochaetes, which are of periplastic or of surface origin. In some species the flagellum may be seen to be made up of two parts—an outer periplastic sheath and an inner fibril or axial filament (Fig. 38). The latter is formed as an outgrowth of a special

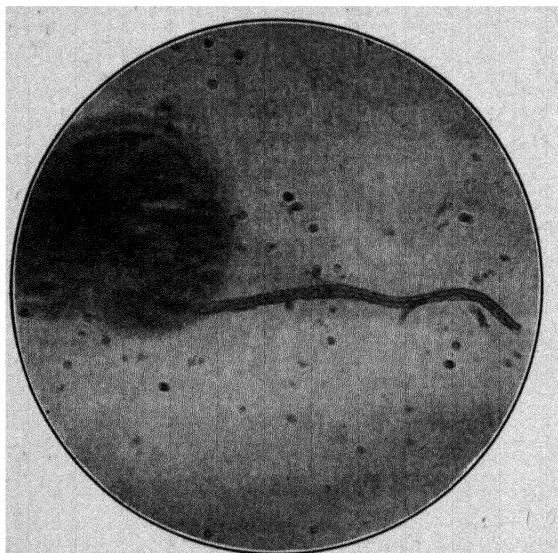


Fig. 38—FLAGELLUM  
STRUCTURE

An unusual photograph showing the axial filament and periplastic sheath of the flagellum of a species of *Bodo*

From a photomicrograph by  
the author

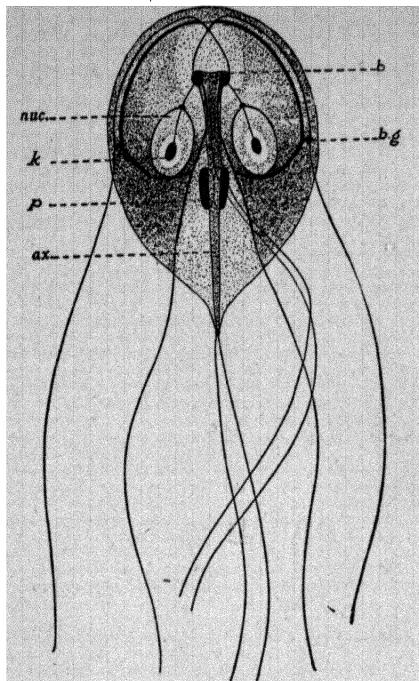
Magnification, 850

See also Fig. 19, page 44

kind of granule, known as a basal body, lying well within the cell, or sometimes within the nucleus. Basal bodies, in turn, are frequently associated with other similarly specialized granules which, from their connection with the locomotor organs of the cell, are called *kinetic elements*, and these in many cases form highly complicated systems (Fig. 39).

Variations in the derived organization of flagellates have to do mainly with: (1) the consistency of the protoplasm and form of the cell; (2) the number and position of the flagella; and (3) the mode of reproduction by division.

In many cases the cortical protoplasm is relatively fluid, so that, by reason of forces generated within, the contour is constantly changing or protoplasmic processes stream out as pseudopodia (false feet). In some types a collar of clear protoplasm

Fig. 39—*GIARDIA MURIS*

A type of intestinal parasite common in man and mammals; a complicated system of kinetic elements in the form of granules and fibers. *ax*, axostyle; *b*, blepharoplast; *bg*, basal body; *k*, endosome; *p*, parabasal body; *nuc*, nucleus

*After Kofoed and Swezy*  
*Magnification, 2100*

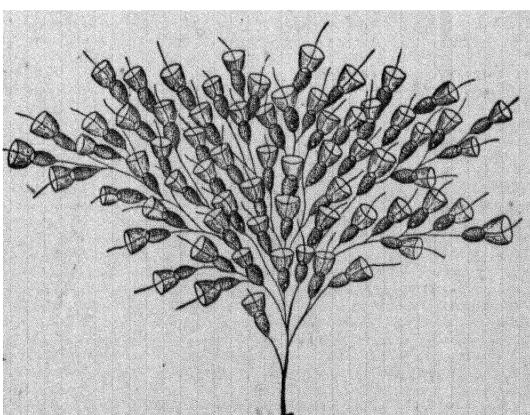
(Fig. 40) is raised up about the base of the flagellum, thus giving rise to cells of the same character as the choanocytes, or collared cells, which make up a large part of the tissues of sponges.

Flagella are highly variable in size and in number. In the simpler forms one is the rule, but forms with two, three, four,

Fig. 40—AN ARBOROID COLONY CALLED *CODONOSIGA CYMOSA*

Each individual bears a conical collar about the flagellum. Similar collar-bearing cells make up a large part of the structure of sponges

*After Kent*  
*Magnification, 400*



six, eight, and many flagella are common. If two are present they may be of the same size, *e.g.*, *Amphimonads*, or of different sizes, *e.g.*, *Monads* and *Bodos*; one may be directed to the front

and the other to the side or the rear, *e.g.*, Prowazekia and Bodos (Fig. 41). A flagellum may pass through the protoplasm to emerge at the rear end, or it may be retained by the periplast

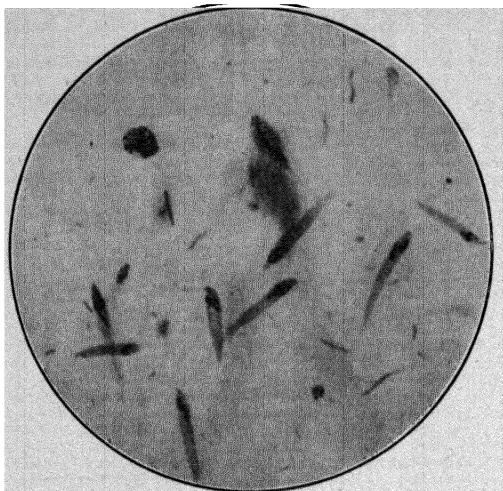


Fig. 41—MISCELLANEOUS PARASITES FROM THE INTESTINE OF A NEWT

Bacteria, spirochaetes, and flagellated protozoa can be made out

From a photomicrograph by the author  
Magnification, 600

and so give rise to an *undulating membrane* (Fig. 42). Multiple flagella are typical of parasitic forms.

#### REPRODUCTION AMONG FLAGELLATES

Reproduction of typical flagellates is by longitudinal division, including division of the nucleus and, in many cases, of the kinetic

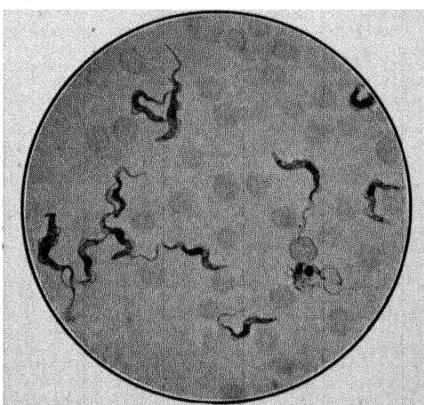


Fig. 42—TRYPANOSOMA GAMBIENSE

A dreaded menace in Africa. These flagellated protozoa cause African sleeping sickness by clogging up arteries and lymph spaces at the base of the brain. Each individual has an undulating membrane which ends in a free whip.

From a photomicrograph by the author  
Magnification, 350

elements as well. In other cases the flagella and kinetic elements are absorbed into the protoplasm and from an *endobasal body* lying within the nucleus new kinetic elements and flagella are formed for each of the daughter cells. In some types, particu-

larly in parasitic forms, the nucleus and kinetic elements may divide a number of times before division of the cell protoplasm, so that when the latter does divide a brood of daughter cells results. Such a multinucleated cell is called a *somatella*. Some unusual types of flagellates possessing two nuclei, *e.g.*, *Giardia* (Fig. 39), or many nuclei, *e.g.*, *Calonympha*, have arisen, apparently by the development of flagella, before cell division of the somatella has taken place and are interpreted as representing permanent somatellae.

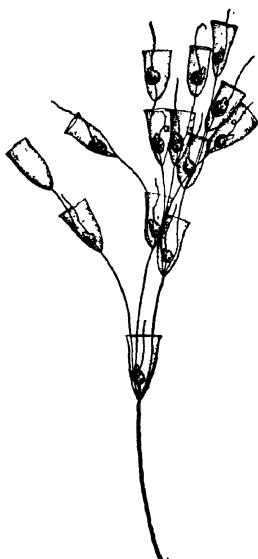


Fig. 43—AN ARBOROID COLONY OF MINUTE ANIMAL FLAGELLATES, *POTERIODENDRON PETIOLATUM*

Each cell is attached by a stalk in its individual cup, and each cup is attached by a stalk to the inside of a parent cup

*From a drawing by the author*  
*Magnification, 410*

Many of the animal flagellates, like the plant flagellates, form colonies as a result of incomplete separation at division or by association in a common gelatinous matrix after division. Individuals frequently grow out on long stalks which remain attached and which expand into cups, within which the monads are fixed (Fig. 43). Or the minute cells may secrete gelatinous tubes, one for each monad, which may lie parallel to one another or which may diverge at varying angles. One such colonial form, *Rhipidodendron*, which is quite common in stagnant waters, is made up of parallel or slightly diverging gelatinous tubes colored brown or red by iron oxide. Because it has a single monad at the extremity of each tube, and because of the parallel arrangement of the tubes, it is called the organ-pipe monad.

Some forms (*Hypermastigidae*) have more than a hundred flagella. The *Hypermastigidae* are interesting because without them white ants cannot live (see page 102).

## THE SARCODINA

*Characteristics*

The Sarcodina type of protozoa is not as definite as the flagellate type, although there are well marked characteristics common to the entire group. The protoplasmic body is rarely restrained by a firm membrane, and streaming movements, whereby portions of the body substance flow outward as pseudopodia,\* are characteristic. There are, however, different types of pseudopodia. Some of them are delicate and needle-like and resemble flagella in having an axial filament and a protoplasmic sheath (axopodia). These are typical of the sun-animalculae (Heliozoa), and of the marine Radiolaria, which comprise the largest group of known protozoa. Another type of pseudopodia, called myxopodia, are much less rigid, and their protoplasm has a remarkable miscibility,<sup>†</sup> such that two or more pseudopodia of the same organism, on coming in contact, fuse to form great nets of protoplasm, which act as food-traps for catching other kinds of minute organisms. This type is represented by the Foraminifera (Fig. 44), another great group of marine protozoa, second in importance only to the Radiolaria.

*Rhizopods*

In fresh water the commonest sarcodina are the rhizopods. These are naked forms such as Ameba, or shell-bearing forms like Arcella, Difflugia, Euglypha, etc. The shells are interesting in that they are frequently composed of minute foreign particles, such as sand crystals, diatom shells, or even of living algae, which are cemented onto the outside of a shell membrane. In all cases this membrane is composed of chitin<sup>†</sup> or pseudo-chitin, which is derived from the protoplasmic protein. In Arcella it forms the shell without additional plates or particles, but foreign particles as in Difflugia, Centropyxis, etc., or home-made plates and blocks, as in Euglypha, Quadrula, etc., are usually cemented to the outside. In Foraminifera there are two such chitinous membranes,

\* See the Glossary of this book, page 123.

† Miscibility, ability to mix, to blend, to become one.

† Chitin, a white, horny, crystal-less substance forming the harder part of the outer covering of insects, crustaceans, and other kinds of invertebrates.

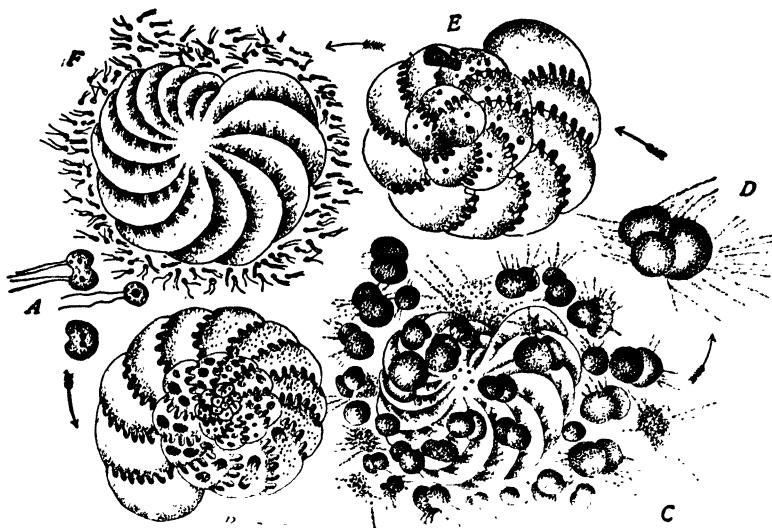


Fig. 44—LIFE HISTORY OF A FORAMINIFERON, *POLYSTOMELLA CRISPA*

Two gametes fuse to form a fertilized cell or zygote, *A*. This develops into an individual with a minute initial chambered shell, *B*. When mature this individual breaks up into a myriad of small ameboid spores, *C* and *D*, each of which develops into a new many-chambered individual, *E*, in which the initial chamber is large. When *E* is mature it breaks up into a cloud of gametes, *F*, which fuse two by two, forming zygotes

*After Schaudinn*

*Magnification, 150*

and calcium carbonate, or lime, is precipitated between these two layers in much the same way as concrete walls are made between frameworks of boards. At the points where pseudopodia extend outward no lime is precipitated; hence pores or windows (foramina) in the shell result, a characteristic which gave rise to the name Foraminifera.

### *Radiolaria\**

In Radiolaria there is no such membrane formation, nor is there a constant deposition of mineral matter. These organisms precipitate silica instead of lime and this glass-like product is laid down in definite skeleton patterns which are often of exquisite design (Fig. 45). The deposition of silica is not a continuous process, but occurs apparently only at periods of saturation, and at such times an entire skeleton is laid down. These periods, known as *dictyotic*<sup>†</sup> moments, may recur a number of

\* See "Fossils," page 23, in this Series.

† Dictyotic, like a net.

times, the last-formed skeleton enclosing those which were formed before. In this way six or more concentric, attached, latticed spheres like Chinese boxes are formed by some types of Radiolaria and remain as a permanent record of the activity of these minute organisms. All Radiolaria, however, do not precipitate silica; some of them manufacture a horn-like compound made of strontium sulphate, with quite a different, but always a complex type of skeleton.

Limestone shells of Foraminifera and skeletons of Radiolaria are constantly raining onto the sea bottom and enormous beds of foraminiferal and radiolarial ooze have accumulated there. Such beds have been cast up from time to time in the geologic history of the earth and have given rise to great tracts of limestone rock, as in the chalk cliffs of England, or to silicious earth, such as that composing the Barbadoes and other islands of the West Indies.

#### *Reproduction Among the Sarcodina*

In structure, the rhizopod Amoeba proteus, with its pseudopodia forming at any point, is one of the simplest types of protozoa, and its constantly changing shape has always been a fascination to the microscopist. It reproduces by cell division after the pseudopodia are drawn in and waste and foreign matters have been extruded. Division becomes a bit more complicated in shell-bearing types. Here, preparatory to division, the protoplasmic body swells by absorption of water and a large portion of the protoplasm protrudes from the shell mouth. This bud assumes the form of the parent, secretes a membrane of chitin, develops pseudopodia, and, after the parent nucleus has divided and provided the bud with one, breaks away from the parent organism. It is a process of budding division analogous to that in diatoms and desmids.

In Foraminifera, when the organism is fully developed, its protoplasm breaks up into a multitude of minute ameba-like cells, a process to which the term *sporulation* is applied. Each of these spores settles down after a short period of swarming and develops a new organism similar to the parent with the exception that the first shell chamber formed is larger than the first chamber of the parent organism. Thus two types of individuals arise,

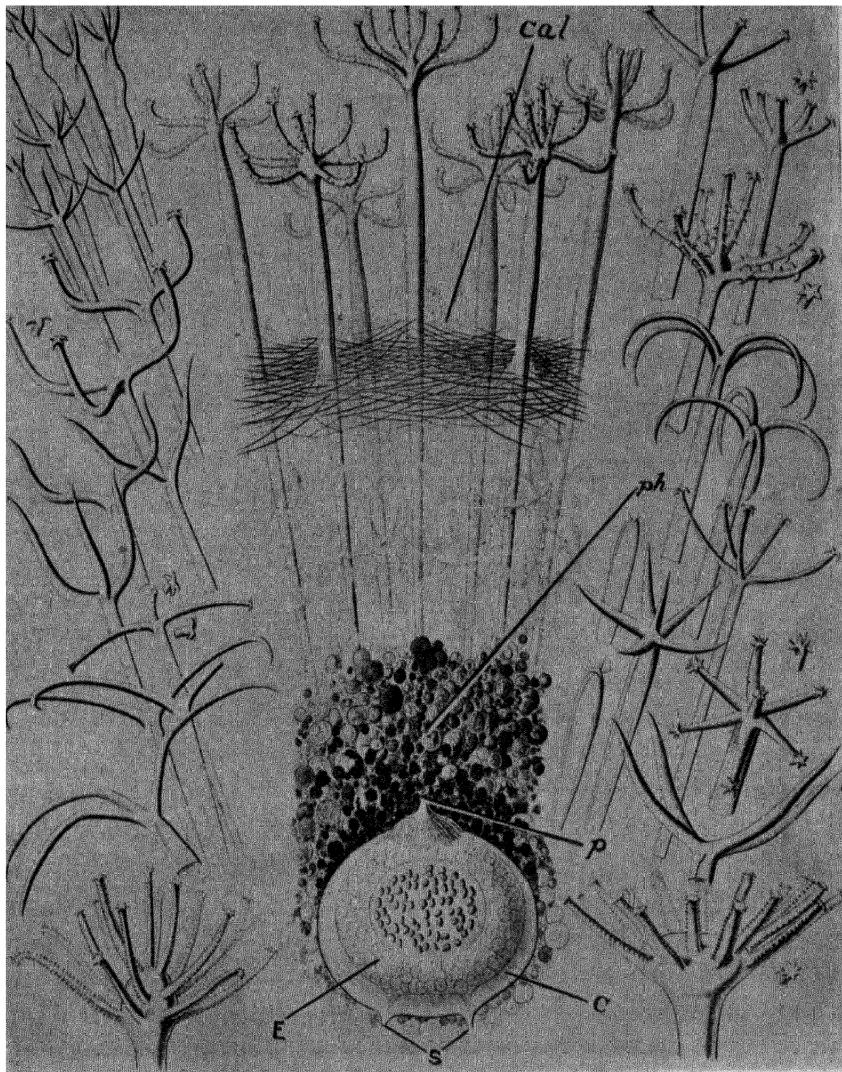


Fig. 45—AULOGRAPHIS SPECIES

Sector of one of the complex Radiolaria belonging to the Legion Tripylea, or forms with three openings (pylea) into the central capsule. *Cal*, calymma or outer veil; *C*, central capsule; *E*, endoplasm with nucleus; *P* and *S*, primary and secondary openings into central capsule; *Ph*, phaeodium or nitrogenous waste; spicules of different shapes are embedded throughout the protoplasm

From "The Voyage of the Challenger"  
Highly magnified

one type with a small central (microspheric) chamber, the other with a large central (macrospheric) chamber (see Fig. 44, page 77). The first type gives rise to asexually produced spores—the second type gives rise to gametes which fuse two by two, thus forming fertilized cells (zygotes) which develop into the microspheric generation. The phrase *alternation of generations* is used to describe this phenomenon—an asexual generation alternating with a sexual generation.

## THE INFUSORIA

### *Characteristics*

In the infusoria\* we find the most highly specialized types of protozoa and the most complex forms of the single cell. Infusoria are found everywhere—in ponds, lakes, seas, in short, wherever moisture is present. The derived organizations, while distinctly of one type, are highly varied, but each is always adapted to its mode of life. Forms, movements, habits are always interesting and frequently spectacular, making them a constant fascination to the microscopist.

### *Cilia*

The most characteristic feature distinguishing infusoria from all other protozoa is the possession of cilia. These are shorter than flagella, are like an eyelash in form, and propel the organism through the water by the synchronous beating of many, after the manner in which the oars of the Roman galleys were operated. In one division of infusoria—the Suctoria—cilia are present in the embryonic stages only, the derived organization of the adult being characterized by the possession of suctorial tentacles† through which food is taken into the body. In the other group—the Ciliata (Ciliates)—cilia are present throughout life.

The distribution of cilia on the body follows fairly definite modifications of type and affords a means of classification of the ciliates. Furthermore, fusion or coalescence of cilia gives rise to motile organs of considerable complexity. In a generalized type the cilia run in longitudinal rows from the mouth at one end to

\* The singular of "infusoria" is "infusorium," and the adjective is "infusorian."

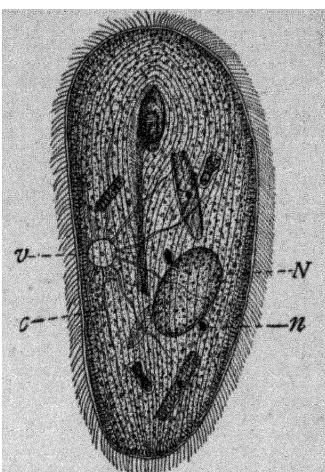
† Elongated processes which are equipped for sucking.

the opposite end of the body (Fig. 46). By coalescence along such rows, usually in the neighborhood of the mouth, undulating membranes are formed. By coalescence of two or more trans-

Fig. 46—FRONTONIA LEUCAS

A typical ciliated protozoan with cilia distributed uniformly about the body. It has a large mouth through which diatoms are taken in. *c*, one of the canals running to the contractile vacuole, *v*; *N*, macronucleus; *n*, micronucleus

From a drawing by Schewiakow  
Magnification, 190



verse rows in the region of the mouth, pyramid-shaped organs, called membranelles, are formed; these are active in food-getting. Since these organs are always present in a curved row leading to the mouth, the aggregate is spoken of as the adoral\* zone of membranelles, or more simply as the adoral zone. Other coalesced cilia may be found on various parts of the ventral surface. These are usually circular in cross-section and are known as cirri† (*singular*, cirrus), specially designated as frontal cirri, ventral cirri, anal cirri, and caudal cirri, according to their location.

Co-ordinating fibrils connect the bases of longitudinal and transverse rows of cilia. Stimuli from the anterior end pass down these fibrils, stimulating the cilia one after the other to contract and resulting in a wave-like contraction of the ciliary coating from the mouth to the opposite end of the body (Fig. 46). In many forms a mechanism resembling a simple nervous system is present. In *Euplotes patella*, for example, fibrils from the posterior anal cirri meet at the anterior end of the body in a structure called the *motorium*, and from this another fibril makes a circuit of the adoral membranelles (Fig. 47). By means of such mechanisms the various activities of the cilia and cilia combina-

\* Adoral, from *ad*, near, and *oral*, mouth—near the mouth.

† Cirri, from the Latin *cirrus*, meaning a curl, a ringlet.

tions are correlated and the organism acts as a unit. Each individual is attuned to a certain "motor response" to any stimulus from the environment. If the stimulus is applied to some local part of the organism the part stimulated does not respond locally,

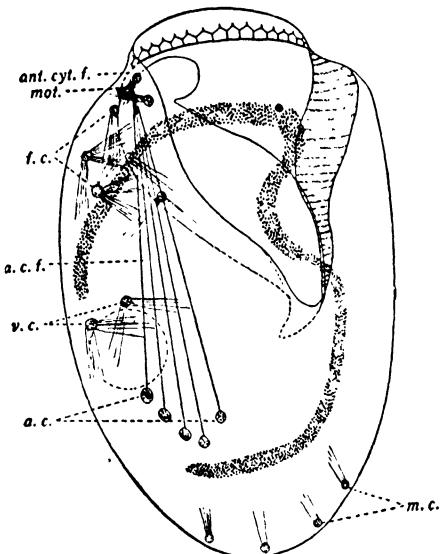


Fig. 47—THE NEURO-MOTOR SYSTEM OF EUPLOTES PATELLA

*a.c.*, position of the anal cirri; *a.c.f.*, co-ordinating fibers from anal cirri to motorium, *mot.*; *ant. cyt. f.*, co-ordinating fibril to anterior lip and down to the mouth. Fibers from the ventral cirri, *v.c.*; marginal cirri, *m.c.*; and frontal cirri, *f.c.*, do not connect with the motorium but end blindly in the protoplasm

*After Taylor*  
*Magnification, 510*

but the stimulus is transmitted to all parts and a characteristic response on the part of the organism results, regardless of the nature of the stimulus.

### *Nuclei*

Another structural characteristic distinguishes the derived adult organization of infusoria from all other protozoa. This is the presence of two kinds of nuclei, one large and of highly varied shapes, the other small and spheroidal; the former is called the macronucleus, the latter the micronucleus (see Figures 46-48). The larger one functions in vegetative activities, the smaller one in cell division and in sexual processes.

### *Cell Division*

Reproduction occurs by transverse cell division, during which the complex structures of the derived organization are resorbed\* and new ones are formed by each daughter half (Fig. 49). Divi-

\* Resorbed, drawn or sucked in.

sion thus results in a reorganization of the protoplasm whereby the motile organs are maintained in a proper proportion to the

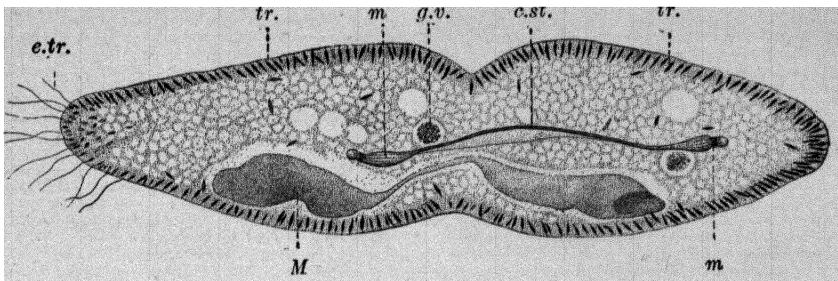


Fig. 48—ALL REPRODUCTION IN THE LONG RUN IS CELL DIVISION

Paramecium caudatum was caught while dividing, killed, embedded in paraffine and sectioned. Macronucleus, *M*, and micronucleus, *m*, are both drawn out but not quite divided; *tr.*, trichocysts, some of which are exploded; *g.v.*, gastric vacuole filled with bacteria in process of digestion; *e.tr.*, extruded trichocyst; *c.st.*, connecting strand

From a drawing by the author

Magnification, 500

size of the young individuals (Fig. 49). In many cases such reorganization is accompanied by profound changes in the macronucleus. *Uroleptus mobilis*, for example, has eight macronuclei, each of which discards about one-third of its substance

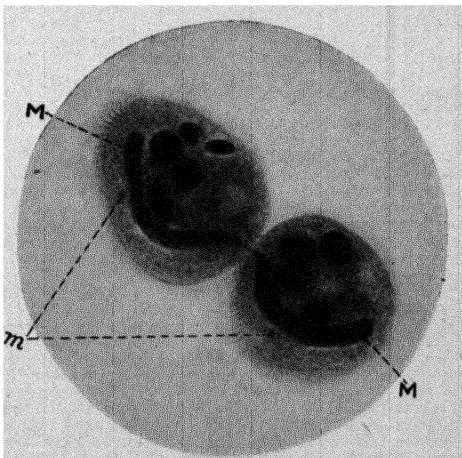


Fig. 49—EUPLOTES PATELLA IN DIVISION

Cell division is a complicated phenomenon. All of the essential structures of the cell divide; the motile organs are resorbed, and formed anew in smaller size, one set for each daughter cell. The photograph shows an end stage of division with the two parts of the macronucleus, *M M*, still connected. The micronucleus *m* has divided

From a photomicrograph by the author

Magnification, 340

into the cell body whereby a new supply of complex nucleo-proteins is provided for activities in the cytoplasm (Fig. 18, page 37). The remaining portions of the old macronuclei then fuse to form a single nucleus, which, with the old micronuclei, now divides (Fig. 50). Thus, cell division, in addition to the function

of propagation, serves as a renovating process whereby old structures are discarded and new ones are formed from the funda-

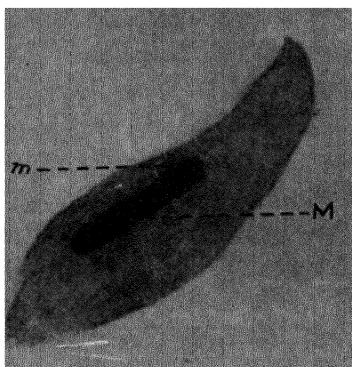


Fig. 50—REPRODUCTION IS A  
RENOVATION PROCESS

*Uroleptus mobilis* normally has eight macronuclei; prior to division these nuclei fuse into one; this then divides to form eight nuclei in each of the daughter cells. *M*, macronucleus; *m*, micronucleus

From a photomicrograph by the author  
Magnification, 600

mental organization. With this renovation there is a renewed potential of vitality, a characteristic of all young organisms.

Cell division in some forms of ciliates, as in some flagellates, results in incomplete separation of the daughter individuals, thus giving rise to colonies. Such aggregates of individuals, colored green by symbiotic algae\* (*chlorella*), may be enclosed in a common jelly, as in *Ophrydium versatile*, which in streams of cold water may form jelly-masses as much as a foot in diameter. Or the daughter individuals may remain connected by stalks and form arboroid colonies, such as *Carchesium*, *Epistylis*, and *Zoöthamnium* (Fig. 51). Muscle-like fibres from the individuals

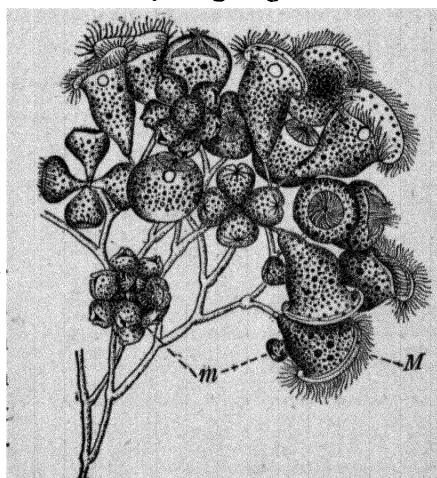


Fig. 51—AN ARBOROID COLONY OF  
EPISTYLIS UMBELLARIA

This is one of the vorticella-like ciliated protozoa. Some, *m*, of the individuals are males; some, *M*, are females

After Greeff  
Magnification, 160

run down the stalks and the main trunk of *Zoöthamnium* so that a single stimulus causes a contraction of the entire colony. Similar fibers traverse the individual stalks but not the main

\* See page 58.

trunk of *Carchesium*, while even stalk fibers are absent in *Epistylis*.

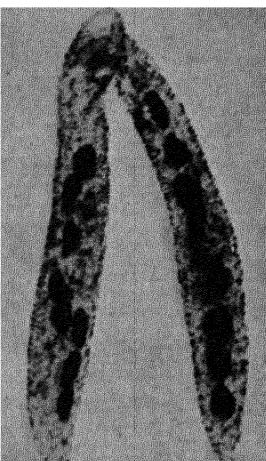
### Conjugation

Another characteristic of the infusoria is the peculiar method of fertilization by conjugation. Two individuals come together and unite at some limited portion, usually in the region about the mouth, thus forming a protoplasmic bridge between the two organisms (Fig. 52). They remain thus fused for approximately

Fig. 52—CONJUGATION STAGE OF *UROLEPTUS MOBILIS*

The two individuals remain fused in the manner shown for 24 hours when they separate. During this time a complicated series of changes occur in the micronuclei

From a photomicrograph by the author  
Magnification, 600



twenty-four hours, when they separate. In the meantime, however, profound changes occur in the cell body of each conjugant. Three consecutive times the micronucleus in each enlarges and divides, forming eight nuclei. These are the so-called meiotic\* divisions, the result of which is the reduction to one-half of the normal number of chromosomes. Maturation† of germ cells, involving the reduction divisions, is characteristic of all higher animals and plants.‡ It is a remarkable evidence of the unity underlying all life to find similar processes in the simplest one-celled animals. After the third division one of the resulting nuclei in each individual moves through the protoplasmic connecting bridge into the other individual where it fuses with a

\* Meiotic, from the Greek, meaning to make smaller.

† Maturation is the final series of stages in the formation of the egg or the sperm, by which its nucleus is prepared for union.

‡ See illustration on page 49 of "Heredity and Variation" in this Series.

nucleus derived in the same way. Thus fertilization is mutual, and by the union of a wandering nucleus and a stationary nucleus, each of which has one-half the normal number of chromosomes, the normal number is restored in the fused nucleus (amphinucleus). In this way the germ-plasms of two individuals are represented in each amphinucleus—a universal biological phenomenon to which the term *amphimixis* is applied.

In the meantime and after the two conjugants, now known as ex-conjugants, have separated, the old macronuclei break up into fragments, and these, together with the unused micronuclei resulting from the maturation divisions, are resorbed into the cytoplasm. New macronuclei and new micronuclei are formed by division of the amphinuclei and the characteristics of the species are restored.

Here, as at division, a complete reorganization of the derived structures occurs with conjugation. But the housecleaning is more drastic and involves all of the nuclear substances as well as the external structures. The protoplasm is quite made over, and not only is there resorption and distribution of great quantities of nucleoproteins but the later nuclear make-up is quite different by reason of the fusion (amphimixis) of nuclear parts of two individuals. Again, this process results in a high potential of vitality, the characteristic of youth.

#### *Encystment and Endomixis*

Like other protozoa, the ciliates have the ability to encyst, and, protected by the impervious cyst membranes, such encysted forms are able to withstand unfavorable conditions of the environment. There is little or no evidence to indicate that encystment is induced by such adverse conditions, although, theoretically, there is no reason why certain conditions of the environment should not bring about the reactions involved in encystment. It is probable that the phenomena of encystment are due to deep-lying causes in the protoplasm itself and occur only when a certain stage of differentiation has been reached. If this is the case, then under adverse conditions those forms which have already encysted will be protected and others will be destroyed. During the process of encystment a ciliate resorbs its external organs, loses its water, and contracts into a dense ball of proto-

plasm (Figs. 17a and 17b); at the same time its macronucleus breaks up and the fragments are distributed throughout the cytoplasm exactly as in the process of conjugation, while the micronucleus divides and gives rise to a new macronucleus and to new micronuclei. A phenomenon of reorganization, paralleling that of conjugation, and termed *endomixis*, is thus an accompaniment of encystment, and, upon emerging from the cyst, the young organism has a high potential of vitality similar to that of an ex-conjugant.

Some types of ciliates apparently never undergo encystment. Thus, cysts of *Paramecium* are rarely seen, nevertheless it undergoes the reorganization changes which characterize encystment in other ciliates, but without encysting.

These three phenomena—cell division, endomixis, and conjugation—are unquestionably of the greatest importance for the survival of protozoan protoplasm and represent a logical consequence of the differentiation which accompanies continued metabolism. Before discussing the line of argument which this suggests let us glance at the metabolic activities which make up the daily life of a protozoan.

## CHAPTER IX

### FUNCTIONAL ACTIVITIES OF PROTOZOA

**A**LL living things are alike in that they perform certain fundamental activities. These, commonly known as the fundamental or vital functions, are: *irritability, respiration, excretion, nutrition, and reproduction.*\*

Irritability is the response of an organism to stimuli and may be visible to the eye in the form of movement, or it may be molecular and hence invisible. Respiration is the intake of oxygen and the disposal of carbon dioxide. Excretion is the process of ridding the body of poisonous waste products such as carbon dioxide, urea, etc. Nutrition is the aggregate of activities having to do with obtaining food, digesting and assimilating it, and adding it to the protoplasmic substances already present. Reproduction is the means by which a life-form is continued and multiplied. In the long run cell division is the only method of reproduction. This is plainly evident in these primitive forms, but is more obscure in the metazoa (many-celled animals), where phenomena of fertilization are necessary to ensure development.

Each function is an aggregate of activities, some of which can be controlled and measured, but not in equal degree. Nutrition, for example, may be controlled through the food supply, and various stages of inanition† brought about by starvation may be studied. All of these functions operate at the same time, and failure of any one link in the chain of activities constituting these functions leads to death and disintegration. Reproduction of protozoa has been considered in the preceding chapter.

#### IRRITABILITY

Irritability visibly manifests itself by various kinds of movement that are determined by the derived organization. The energy necessary to produce this movement is obtained through

\* See "The Coming and Evolution of Life," page 7, in this Series.

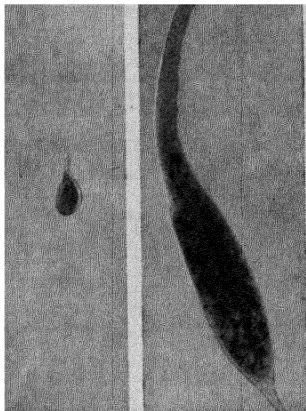
† Inanition, exhaustion from lack of food.

the oxidation or burning of substances in the protoplasm, and for this oxygen is necessary, hence the need of respiration. Nitrogenous waste (urea) and carbon dioxide are products of the continuing breakdown of protoplasmic substances by oxidation and by other chemical processes; they act as poisons and must be disposed of, if protoplasmic activities are to continue unhampered.

Fig. 53—TWO SISTER CELLS OF *DILEPTUS GIGAS*

*B*, a normal individual properly nourished; *A*, an individual, sister cell of *B*, which had been starved for two weeks. Same magnification

From photomicrographs by the author  
Magnification, 65



*A*

*B*

This disposal is effected by the processes of excretion. The continued breakdown of protoplasmic substances leads to a continuous loss of living substance, but the loss is constantly made good through the activities of the function of nutrition. Thus, repair and growth, and finally the vital function of reproduction, follow nutrition.

Let us see how this works out in the case of a starving Paramecium, which normally feeds on the bacteria of infusions. Observations of this nature were first carefully made in 1902 by Professor Hans Thure Sigurd Wallengren, a well-known Swedish naturalist. He kept normal individuals in tubes of pure water and watched them daily for about eleven days. At the end of this time he found that most of them had died and that the few survivors were much vacuolated and abnormal in appearance. After the first day of the experiment they appeared clear and transparent, indicating the loss of their nutritional reserves. After five days small vacuoles (minute cavities filled with a watery fluid) appeared, and these increased in size and number until little more than a skeleton of the organism was left.

With other ciliates the effect of starvation may be simply a reduction in size without the appearance of vacuolization (Fig. 53).

Similarly in all protozoa, as a result of continued movements, the protoplasmic substances are burned up, the first to go being the protein food reserves, after which the basic substances of the protoplasm are used as fuel, a process which, in *Paramecium*, results in great spaces being formed in the organism. Up to a certain stage recovery of the animal is possible by the introduction of food, but beyond this point continued starvation ends in death.

### RESPIRATION

Not much is definitely known about the process of protoplasmic oxidation,\* but attempts have recently been made to determine the intensity of oxidation at any given time by injecting into the protoplasm graded chemical indicators which change color on undergoing oxidation. The depth of color indicates the extent of oxidation undergone.

There are no special respiratory organs in protozoa. Oxygen is taken into the cell with water or possibly by osmosis,† and carbon dioxide ( $\text{CO}_2$ ), is given off in the same way. In all fresh-water protozoa, but not in marine forms, there is a specialized organoid called the contractile vacuole which may function in the disposal of carbon dioxide. This organoid is so named from its rhythmic contractions whereby fluids in the vacuole are expelled from the organism. In some forms this vacuole is a simple spherical cavity which grows steadily in diameter by increments of fluids from the protoplasm until it reaches a maximum size. It is connected with the outside by a fine and generally short canal, which is closed by a delicate protoplasmic film. When the internal pressure of the contained fluid wastes overcomes the resistance of the surrounding film, the latter breaks and the fluid escapes, a new film being formed by the protoplasmic wall of the vacuole.

\* Oxidation, the process of adding oxygen to other chemical elements or to existing chemical combinations.

† Osmosis, the passing of a fluid through a porous partition, such as a membrane, to mix with another fluid on the other side. The inward flow is called *endosmosis*, and the outward flow, *exosmosis*.

## EXCRETION

The contractile vacuole in many protozoa also acts as an organ of excretion, and in many forms it becomes quite complicated. In some a central contractile vacuole is filled by material coming through canals, which act as feeders, leading from various parts of the organism. (See, for example, *Frontonia leucas*, Fig. 46, page 81.) In *Paramecium* there are two vacuoles, one in the anterior third of the body, the other in the posterior third. Leading toward each of them are five or six canals which are quite obvious when filled, but which are difficult to see when emptied. The fluid collected by these canals is poured into the vacuoles, which contract when full. At ordinary temperatures these vacuoles contract about twice a minute.

Opinions as to the function of the contractile vacuole are not unanimous. To the German naturalist, Christian Gottfried Ehrenberg (1795-1876), and other early students of the protozoa, the vacuole appeared as a beating heart and the canals were interpreted as arteries or larger blood vessels. More recently the vacuole has come to be regarded as a means of regulating the water equilibrium between the protoplasm and the surrounding medium. Thus a fresh-water form like *Amoeba crystalligera* or *Amoeba verrucosa* has a contractile vacuole, but if one of these individuals is transferred to sea water the vacuole is lost and the protoplasm becomes much condensed. Conversely, if a parasitic ameba of the oyster which is normally without vacuoles be transferred to fresh water, it develops several of them. It has also been demonstrated that by increasing the density of a fresh-water medium the vacuole becomes smaller and at a certain density disappears. Such experiments indicate a definite physical relationship between the organism and the environment —a relationship which involves the density factors of both the protoplasm and the medium, the permeability of membranes, and the processes of endosmosis and exosmosis.

There is, however, still another probable function that may be attributed to vacuoles. A considerable volume of water passes through the protoplasm of a *Paramecium* in an hour. Here the canal distribution indicates that water is collected from all parts and is ultimately discharged from the vacuole. Any

soluble waste matters in the protoplasm, such as carbon dioxide ( $\text{CO}_2$ ) and nitrogenous waste in the form of uric acid or urea, will be dissolved in and disposed of with the water. Thus the contractile vacuole can be regarded as a primitive excretory system, a conclusion supported experimentally by recent workers who showed that in *Paramecium caudatum* uric acid is actually present and to an amount equal to approximately four to five milligrams per liter of water.

## NUTRITION

The constant movement of ciliated protozoa, involving a constant supply of energy derived through the burning of protoplasmic substances and leading to the constant formation of waste matters which must be excreted, necessitates a constant renewal of substances if the organism is to live. We have seen (page 89) how a *Paramecium* disintegrates if this repair of body material is prevented by starvation. The aggregate of processes concerned in such repair are grouped together under the comprehensive term *nutrition*.

Although the processes concerned with nutrition all operate at the same time and are closely interwoven, we can examine them individually under the headings of (1) food getting, (2) digestion, (3) distribution and assimilation, and (4) defecation.

### *Food Getting*

The methods employed by protozoa to obtain needed materials for repairing waste are all correlated with the phenomena of irritability. The special method used by any one type is the result of many factors of the organization and adaptation to the mode of life of the organism. It is probable that no two types of protozoa employ an identical method. Nevertheless it is possible, and certainly convenient, to group the manifold activities under a few main types, such as, (1) holozoic, (2) saprozoic, (3) holophytic, and (4) mixotrophic.\* These four types are usually given as the different methods of nutrition. The dif-

\* *Holozoic*, wholly or distinctively like an animal as to nutrition; *saprozoic*, living on proteins and carbohydrates in solution; *holophytic*, wholly or distinctively vegetable in nutrition—obtaining food after the manner of a green plant; *mixotrophic*, deriving nourishment from a combination of any two or even of all three of above methods.

ferences between them, however, have mainly to do with the nature of the raw materials taken in by the organism and with the subsequent processes necessary for their assimilation.

Holozoic nutrition in protozoa, as in higher animals, involves the ingestion of food in the form of proteins, carbohydrates, and fats, which are usually obtained from the protoplasm of the other living organisms that are eaten. It is an expensive mode of feeding since it requires labor in the capture and killing of living prey, preparation and secretion of digestive fluids and ferment necessary to dissolve the proteins and carbohydrates, and defecation of the indigestible remains.

Saprozoic nutrition is a more economical method, for in this process the organism does away with the elaborate processes of digestion and relies upon the activities of other organisms to prepare the dissolved proteins and carbohydrates that are ingested. Such types live upon dissolved foods, prepared mainly by the bacteria found in infusions or by the hosts of such forms as are parasitic. The food is taken in through the body wall or through special receptive regions by endosmosis.

Holophytic nutrition, characteristic of the chlorophyll-bearing types, is quite different in principle from the other two. The ingestion of solid or dissolved proteins and carbohydrates is absent. Instead, the very unstable substance, chlorophyll, is manufactured in the presence of light by specialized plastids (chloroplastids) of the cell. Chlorophyll is very sensitive to light and in some way not yet fully understood is instrumental in utilizing the radiant energy of sunlight to bring about the union of carbon, oxygen, and hydrogen in proper proportions to form the group of chemical compounds known as carbohydrates.

Finally, mixotrophic nutrition is characteristic of those forms which combine any of the above methods of acquiring raw materials. Some forms combine holozoic and saprozoic methods, others holozoic and holophytic, still others holophytic and saprozoic, and some combine all three methods.

#### *Continuous Feeders*

The great majority of protozoa are holozoic in their food getting; many of them apparently eat all the time, and according to the French biologist, Émile Maupas (1844-1916) are glut-

tons *par excellence* of the animal kingdom. Others are occasional feeders. Continuous feeders, so called, are those forms of ciliated protozoa with a permanently open mouth toward which a current of water, bearing bacteria and other minute forms, is constantly directed by the action of cilia about the

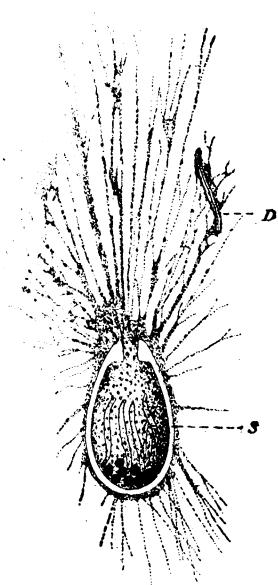


Fig. 54—FOOD GETTING BY TRAPPING

*Allogromia oviforme*, a common marine shore-dwelling foraminiferon with a chitin shell (S) and one mouth-opening through which there is a constant streaming of protoplasm. A diatom, D, has just been captured in the pseudopodial network

After M. Schultze  
Magnification, 50

mouth. After passing through the mouth the water and food are collected in a vacuole which, upon reaching a certain size, is carried away from the region of the mouth by protoplasmic movement. Such vacuoles are known as *gastric vacuoles* or improvised stomachs, for while in them food substances are digested by means of digestive ferments that appear in the vacuole.

#### *Occasional Feeders*

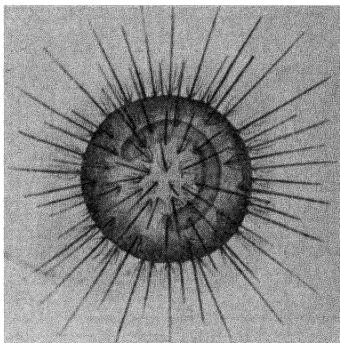
Occasional feeders eat whenever chance brings prey within the radius of their activity, and many of them are guilty of feeding, at times, upon their close relatives! In some species balloon-like membranes are spread out like sails for directing the food currents toward the mouth. In Foraminifera, net-like traps of pseudopodia are spread out for the capture of larvae, diatoms, and smaller protozoa (Fig. 54). The most spectacular forms, however, are those which capture and paralyze living prey

by methods akin to harpooning. Here long tentacles, as in the ciliate *Actinobolus radians* and in *Suctorria*, usually radiate out in the water and are activated by the stimulus of a passing organism. *Actinobolus* is most interesting, for it is stimulated to activity usually by only one type of food organism. This remarkable organism possesses a coating of cilia and protractile tentacles, which may be elongated to a length equal to three times the body-diameter, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts.\* When at rest

Fig. 55—"HARPOON" METHOD OF FOOD GETTING

A ciliated protozoan, *Actinobolus radians*, which lies mouth down, in wait for prey. The ends of the spine-like tentacles hold a bit of poison which is inoculated into a small organism like *Halteria* if it strikes against them. The prey is paralyzed and is then drawn around to the mouth by shortening of the tentacles and action of the cilia

*After Moody*  
*Magnification, 275*



(Fig. 55) the mouth is directed downward, and the tentacles are stretched out in all directions, forming a minute forest of plasmic processes, among which smaller ciliates, such as *Urocentrum*, *Gastrostyla*, etc., or flagellates of all kinds, may become entangled without injury to themselves and without disturbing the *Actinobolus* or drawing out the fatal darts. When, however, an *Halteria grandinella*, with its quick and jerky movements, approaches the spot, the carnivore is not so peaceful. The trichocysts are discharged with unerring aim, and the *Halteria* whirls around in a vigorous, but vain, effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed. The tentacle, with its prey fast attached, is then slowly contracted until the victim is brought to the body, where, by action of the cilia, it is gradually worked around to the mouth and swallowed with one gulp. Within the short time of twenty minutes I have seen an *Actinobolus* thus capture and swallow no less than ten *Halterias*.

\* Trichocysts are minute stinging organs (see Fig. 48, page 83).

Another type is illustrated by the ciliate *Didinium nasutum*. It is a small but powerful organism which darts here and there with an erratic movement, rotating on its axis at the same time. During such sudden darts it may strike a *Paramecium*, or other ciliate. A complex "seizing organ" bearing a dose of poison is buried in the *Paramecium*, which is instantly paralyzed. The seizing organ with prey attached is then drawn into the body of the captor and the *Paramecium* is swallowed whole (Fig. 56). This is quite a gymnastic feat, for the captured *Paramecium* is frequently larger than its captor, and I have often seen a *Didinium* swallow two conjugating individuals at one time!

### *Digestion of Food*

It is a significant fact that food substances engulfed by these ciliates undergo pretty much the same treatment as though they had been taken into the digestive tract of some higher type of animal. Proteins and carbohydrates must be made soluble. In all cases this is accomplished by hydrolysis (splitting of the complex molecules of the solid food through the addition of water), and is brought about through the agency of digestive ferments—amylolytic ferments for transforming solid carbohydrates into soluble sugars and proteolytic ferments for hydrolyzing proteins. In the higher animals there are several kinds of proteolytic ferments, some of which, like the pepsin of the stomach, are active only in an acid medium, while others, like the trypsin of the smaller intestine, can act only in an alkaline medium.

### *Distribution and Assimilation of Food*

In ciliates, particularly in the case of *Paramecium*, the form on which most of the observations have been made, analogous digestive processes occur. Bacteria taken in as food are killed by an acid secreted in the gastric vacuole. The acid reaction persists for a short time (10 to 15 minutes), after which the fluid of the vacuole gives an alkaline reaction. Disintegration of the bacteria occurs in the alkaline medium, and the former bacteria, now changed to the form of minute granules, are distributed throughout the protoplasm.

The acid and alkaline reactions of fluids in gastric vacuoles vary widely in different types of protozoa and even in the same

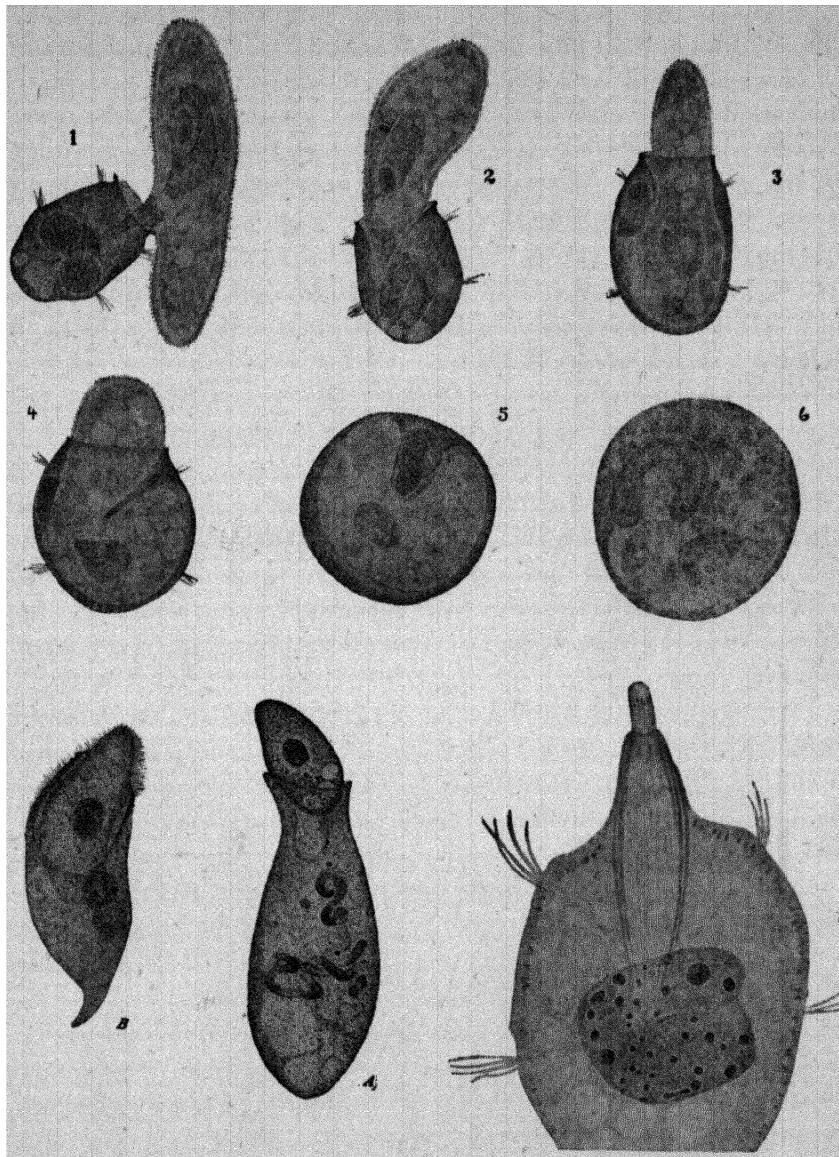


Fig. 56—TABLE MANNERS OF SOME CILIATED PROTOZOA

*A*, *Spathidium spathula* and *B*, *Lionotus fasciola*, both eat the same food and were caught in the act of swallowing a *Colpidium colpoda*. 1 to 6, stages in the act of swallowing a *Paramecium caudatum* by a smaller ciliate, *Didinium nasutum*. 7, a section of *Didinium* showing the internal structure and the seizing organ in the proboscis with which it catches and paralyzes *Paramecium*.

The seizing organ then retracts, drawing the victim in with it (2-4)

From photomicrographs by the author

Magnification, 140

type. In some strains of *Paramecium* an acid reaction persists throughout, and in some forms, *e.g.*, *Actinosphaerium*, active digestion occurs only in an acid medium.

In some cases, the ferments responsible for the digestion of proteins have been isolated by extraction. In 1886 pepsin-like ferments, which will dissolve albumin in an acid medium, were obtained from one of the slime molds, *Ethalium septicum*, and in 1893 from the ameba-like rhizopod, *Pelomyxa*, while trypsin-like ferments, active in an alkaline medium, have been obtained from a number of other forms.

The method of determining the acid or alkaline character of a gastric vacuole is rather interesting. Wilhelm Friedrich von Gleichen, one of the early German physiologists in the eighteenth century, discovered that minute granules of colored substances, such as carmine or indigo which are practically insoluble in water, will be taken into the gastric vacuoles of protozoa as readily as will bacteria. The vacuoles thus become brilliantly colored and remain so through the processes which result in the digestion of ordinary food.

Using this method and seeing one vacuole fill and move away to be followed by another, and this by a third, etc., Ehrenberg, in 1838, concluded that many of the protozoa differ from higher animals in having numerous stomachs, and was led to the creation of a special group for them, the Polygastrica. The same method of experimentation has been more fruitful in the hands of modern observers, who, using substances which change color in both acid and alkaline media, were able to tell the duration of these chemical states. Thus in 1905 Serge Ivanoric Metalnikov, then director of the biological laboratory in the University of St. Petersburg, Russia, used granules of alizarin sulphate, which remain violet in color in the alkaline medium in which *Paramecium* lives, but turn yellow after a short period in the gastric vacuole. A still more refined method has been introduced with the microdissection apparatus.\* By the aid of this instrument delicate chemical indicators in fluid form are injected into

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\* A microdissection apparatus consists of extremely finely drawn glass tubes which are attached to and manipulated by means of micrometer screws with vernier readings. The whole assembly is placed on a microscope stand. In the hands of an expert extremely delicate operations can be performed on individual cells.

the fluids of a vacuole and the color changes afford an index of the degree of acidity.

Similarly with other types of food; ferments capable of changing solid carbohydrates into soluble sugars have been extracted from different types of protozoa, but experimenters have not been equally successful in demonstrating the presence of fat emulsifying ferments.

From all the observations and experiments it appears, then, that the repair of metabolic waste involves activities of a complicated nature, including the capture and swallowing of food and the transformation of that food from the condition of solids to a condition of soluble proteins and sugars. Just how even these rather simple proteins, for example, amino acids, are assimilated or added to the living protoplasmic substances is still unknown, but the fact is easily demonstrated that in an incredibly short time the specific protein of a Paramecium, for example, becomes the equally specific but different protein of a Didinium. Thus, if a single individual of Didinium is placed in a drop of water with eight individuals of Paramecium, in twenty-four

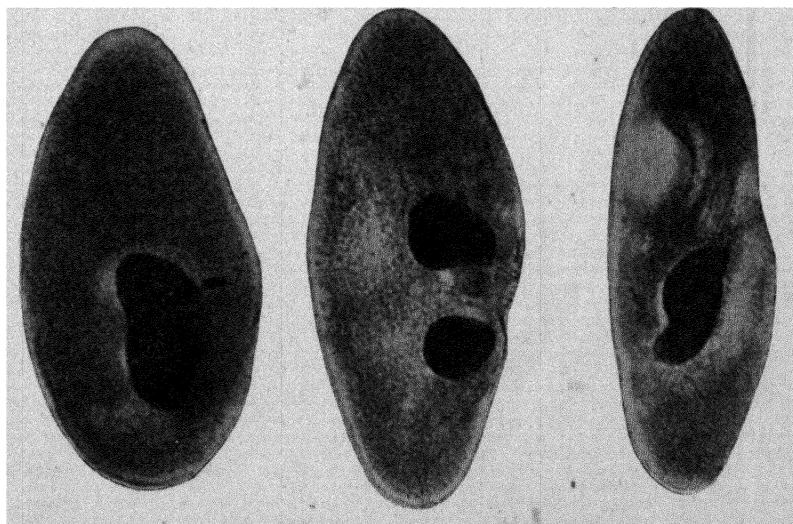


Fig. 57—PROTOZOA IN NEED OF A DOCTOR

*A*, a *Paramecium caudatum*, which has eaten too long and too much of the same kind of food and has become semi-solid; it would die if not treated. *B* and *C*, two individuals which were in this condition, but were treated with dilute potassium phosphate, and are again becoming soft.

*From photomicrographs by the author*

*Magnification, 430*

hours there will be eight individuals of Didinium and not one Paramecium!

It occasionally happens, especially with forms that have been maintained for long periods on the same diet, that the protoplasm becomes loaded with unassimilated food reserves or with the waste products of metabolism. Figure 57 illustrates a Paramecium in such a condition; in this case it was restored to normal condition by a brief treatment with potassium phosphate and with the salts contained in a dilute solution of beef extract.

#### DEFECATION

Defecation is the function of eliminating from the body substances which cannot be digested. In the rhizopod types there is no definite spot where such elimination occurs, but in the more complex infusoria a definite anal opening is present (Fig. 3, page 10).

## CHAPTER X

# PARASITISM AMONG PROTOZOA

### ADAPTATION TO ENVIRONMENT

THE multitude of substances making up protoplasm are always in a physico-chemical condition of delicate equilibrium. Adjustments are easily made to the ordinary stimuli from the environment, and these adjustments form a part of the daily life of an organism in food-getting, respiration and excretion, avoiding enemies, and the like. An unusual environmental stimulus constitutes something of a shock, and adjustment to it depends upon the nature and the intensity of the stimulus. Heat, for example, accelerates all activities and the resultant reactions conform in a general way with Van't Hoff's law to the effect that a reaction doubles with an increase of ten degrees in temperature. Too great a heat-shock kills, but a succession of smaller shocks builds up a heat-resistance until the organism becomes adapted to the ordinary lethal or fatal dose. Thus William H. Dallinger, an English experimenter, found that flagellates of the genus *Dallingeria* (*Heteromita*), living in a normal temperature of 60° Fahrenheit, were killed by raising the temperature to 78°, but if the temperature was slowly increased, with sufficient time for the organisms to become adapted to the increments, they could be subjected to 78° without harm. He found that some individuals could be trained to live normally in a temperature of 158° Fahrenheit, but it required seven years to bring this about. Similarly, an *Amoeba verrucosa* living normally in fresh water may be transplanted to salt water and still live, but the transfer involves a marked change in structure—the contractile vacuole disappears entirely, much of the protoplasmic water is lost, and the ameba becomes much denser and smaller than it is normally. Various types of poisons—arsenic, strychnine, mercury, quinine—are lethal to protozoa, but resistance to these poisons may be built up in the organism in the same way

as is heat-resistance. This has an importance in connection with certain types of human disease. Malaria organisms, for example, may become adapted to quinine poisoning and be able to resist large doses of the drug. Many pathologists regard the malarial relapse as due to such "quinine-fast" organisms, which become virulent under certain conditions of the blood.

The irritability of protoplasm, together with its power of adjustment or adaptation, underlies all parasitism. Together with water and food, protozoa are constantly being taken into the digestive tracts of higher animals. In the stomach they enter a denser and an acid medium in which the vast majority of such water-dwelling forms are quickly killed. Some protozoa, particularly those already adjusted to a denser medium, are better able to withstand the hardships of the new environment. Such resistant forms may be the making of parasites. But they must be adapted also to withstand the action of proteolytic ferments which operate in the alkaline medium of the intestines. Such resistant forms are said to be immune to digestive fluids, and they may or may not become the causes of diseases, the result of the association of the host and the parasite being dependent upon the nature of their adaptations. In general, we may say that the more recent the association of host and parasite, the more apt is the result to be pathogenic. For example: the blood disease known as nagana, which attacks the cattle of southern Africa, and which is due to the flagellate, *Trypanosoma brucei*, is only mildly harmful to the native cattle but is quickly fatal to European cattle.

#### ASSOCIATIONS FOR MUTUAL GOOD

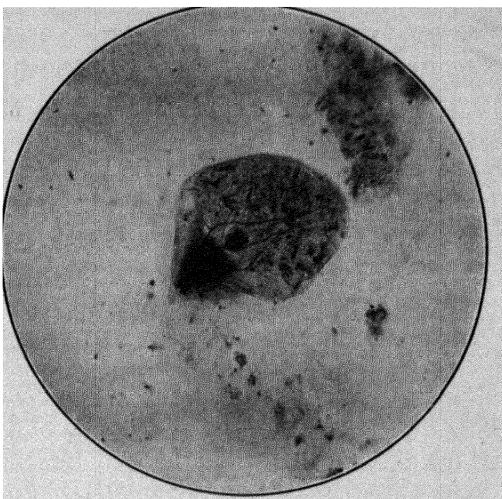
Great numbers of protozoa have become adapted to life in the cavities, fluids, and tissues of higher animals. The digestive tract in particular is a center of activity for such adapted forms, and no animal is exempt. In some cases these protozoa are harmless guests, in which case they are called *commensals*, or they may be even beneficial to the host by keeping down the growth of bacteria. In some cases, rare indeed, they have made themselves indispensable to their host by preparing the food on which the latter is dependent. Thus the highly complex flagellates, known as *Hypermastigidae*, *e.g.*, *Trichonympha* (Fig. 58), in-

habiting the lower intestine of white ants (termites), transform the wood eaten by a termite into soluble sugars on which the termite, as well as its guests, thrive. Ingenious experiments by

Fig. 58—A WORKING PARTNER

*Trichonympha campanula*, a complex flagellated protozoan which changes solid wood into soluble sugar for white ants

*From a photomicrograph by the author  
Magnification, 150*



the American biologist, Dr. L. R. Cleveland (1892- ) have demonstrated that termites if deprived of these associates die of starvation.

### HARMFUL ASSOCIATIONS

There is no doubt that many kinds of protozoa in the intestines live by absorption of food digested by the host and to this extent rob the host of nutriment, but when compared with the food absorbed by a tapeworm, for instance, such loss is negligible. A more serious situation is provided by those forms which lie among the cells lining the wall of the intestine and which break down the functioning cells of the host by secreting their own digestive fluids. In this way *Endamoeba dysenteriae* causes the ulcers and abscesses in the intestines and liver which are characteristic of amebic dysentery.

Once adapted to the conditions of the digestive tract, further adaptations which may lead to progressive parasitism are always possible. Thus the flagellates *Herpetomonas* and *Lepomonas* are harmless parasites of the digestive tracts of lower animals, but a closely related genus, *Crithidia*, has progressed a step farther in parasitism and plants itself in great numbers on

the surface of the secreting cells and thus interferes with the normal functioning of the intestines. A further step has been made by the genus, *Leishmania*, which invades a cell and multiplies there until a single liver cell may contain as many as three hundred individuals. This genus is responsible for very serious human diseases—the kala azar, or black disease, of India, the infantile ulcer of Mediterranean countries, and the loathsome leishmaniasis, or espundia, of Brazil. Similarly, the coccidian parasite, *Cyclospora karyolytica*, invades and kills the epithelial cells of the ground-mole. Great areas of functional tissue are destroyed, and, according to Schaudinn, the mortality of infected moles is 100 percent (see page 106).

### MOVING TO A NEW HOST

Progressive parasitism is shown in still another way in the cases of parasites which have become adapted to life in lower cold-blooded animals, such as flies, mosquitoes, fleas, leeches, etc., which in turn prey upon higher types of animals, usually as blood suckers. Their parasites may thus be introduced into the blood stream of the victim, and if such parasites have the power to adapt themselves to the novel thermal and chemical conditions of the new environment they continue to live. In this way human diseases, like African sleeping sickness, and many diseases of domesticated animals, are contracted from biting flies; malaria of man and birds, yellow fever, and infectious jaundice from mosquitoes; Chagas disease from biting bugs; spirochaetoses of different kinds from ticks, etc. The effects on these secondary mammalian hosts differ in different cases. Species of the genus *Plasmodium*, causing malaria, upon reproduction break down enormous numbers of red blood corpuscles and at the same time liberate poisons which cause the characteristic symptoms of chill and fever, the regular periodicity of reproduction of the parasite giving the typical recurrent attacks of fever. Great quantities of freed hemoglobin, the coloring matter from destroyed red blood corpuscles, overtax the ability of the liver to transform it into bile, the surplus is carried to the kidneys, and thus hemoglobinuria is induced as a secondary symptom of malaria.

In other cases the mammalian parasites play a more passive

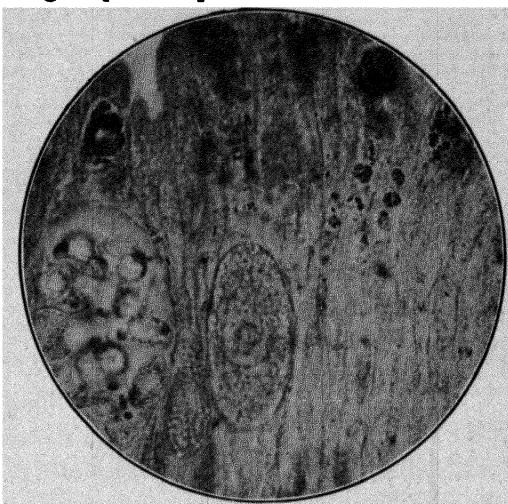
rôle, as in African sleeping sickness where the Trypanosomes (Fig. 42, page 74) accumulate in the lymphatics, and, together with attracted white blood corpuscles, block up the capillaries and smaller arteries. When this happens in the base of the brain, starvation and atrophy of the brain cells accompanied by their loss of function result. This, in turn, is manifested by characteristic lethargy, coma, and finally death.

Representatives of all groups of protozoa have become

Fig. 59—*ADELEA OVATA*

A full-grown coccidian in an epithelial cell of the intestine of the centipede. The nucleus with its endosome is distinct. At the left is a group of spores each with two sporozoites

*From a photomicrograph by the author  
Magnification, 600*



adapted to parasitism and few animal types are free from them; even protozoa themselves are subject to such infections! Parasitic flagellates and ciliates are widely distributed, the latter particularly in cattle and horses; rhizopods, with the exception of ameba, are poorly represented among the parasites.

### SPOROZOA

In addition to the parasitic forms so far described, and also in addition to the three types of protozoa—Mastigophora, Sarcodina, and Infusoria—described in Chapter VIII there is a fourth type of protozoa—the Sporozoa—made up of obligatory parasites, not a free-living representative being known. These forms are without motile organs. They reproduce for the most part by means of encapsulated\* spores, whereby protection is assured against external conditions in passing from host to host.

\* Encapsulated, inclosed in a membrane or capsule.

One great group of Sporozoa, Gregarinida, are parasites of digestive tracts and body cavities of invertebrate animals; another great group Coccidia (Fig. 59), are parasitic in the epithelial cells of both vertebrates and invertebrates; others are parasites of the blood stream, *e.g.*, Haemosporidia (Fig. 60),

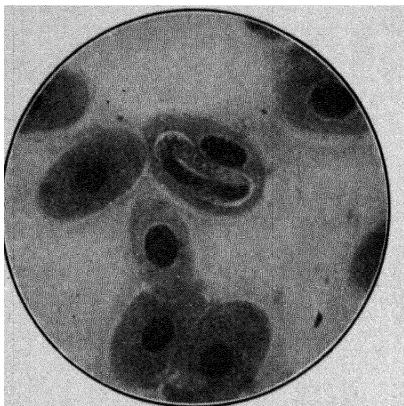


Fig. 60—A PARASITE, HAEMOGREGARINA STEPANOWI, IN A BLOOD CELL OF A TURTLE

This organism always curls up in this characteristic way alongside the nucleus

From a photomicrograph by the author  
Magnification, 700

and still others of tissues, *e.g.*, Neosporida. Gregarines are apparently harmless to their hosts, but the other Sporozoa, with their cell-destroying habits, are dangerous, frequently causing disastrous epidemics both among men and among domesticated animals, and are dreaded enemies of the breeders of fishes, silk-worms, and bees.

The life history of a typical sporozoan is definite, and with some variations may be applied to all forms. I will illustrate with *Eimeria schubergi* (Fig. 61), an intestinal parasite of the common centipede and belonging to the same group as the form which is fatal to all ground-moles (see page 104). The young germ, known as a "sporozoite," is freed from its capsule by the solvent action of the digestive fluids of the centipede. It penetrates an epithelial cell and grows into a large intracellular parasite, the nucleus of the infected cell being pressed to one side where it atrophies. The nucleus of the parasite meanwhile divides several times, after which its cell body breaks up into as many small cells as there are nuclei. These small cells, known as agametes (Fig. 62), or asexual spores, break out of the epithelial cell and penetrate other normal epithelial cells in which the process of growth and sporulation is repeated. This is

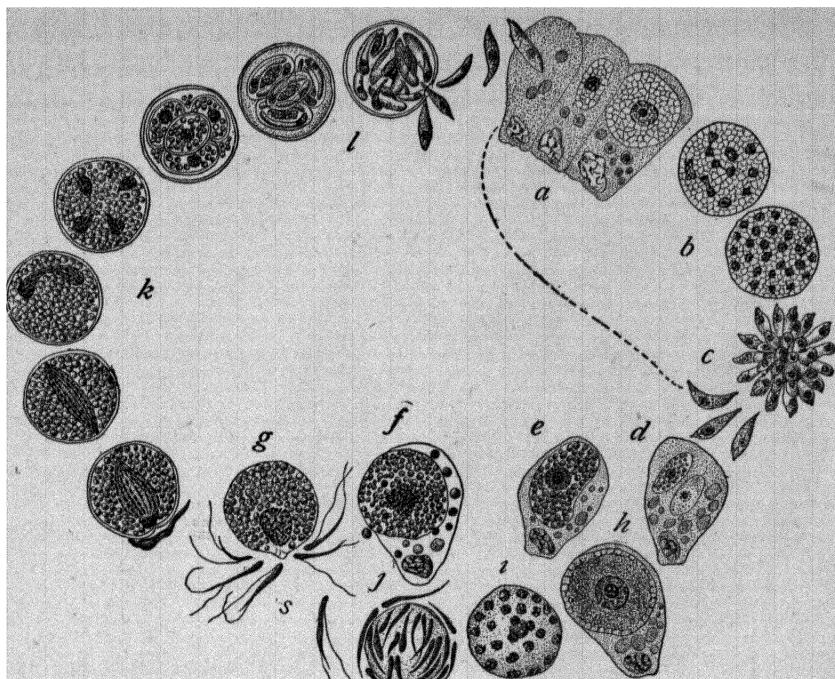


Fig. 61—A TYPICAL LIFE CYCLE OF A SPOROZOAN—  
*EIMERIA SCHUBERGI*

A germ, called a sporozoite, enters an epithelial cell, *a*, and grows to a large intra-cellular organism; its nucleus divides, *b*, and the cell breaks up into many spores, *c*. These enter new cells and repeat the cycle. Ultimately two different kinds of individuals are formed by the spores. One kind (*d,e,f*) is large and has a heavy, granular protoplasm; this is a female type or macrogamete, *g*. The other type grows into a transparent cell which forms a multitude of very fine sperm-like cells, the microgametes (*h,i,j,s*). The egg is fertilized by a microgamete. This is followed by an entirely different kind of sporulation. The fertilized nucleus divides twice and four cells are formed, each having a capsule about it (*k,l*). Within each of these, two germs, or sporozoites, are formed, and these in a new host, repeat the cycle

*After Schaudinn*

*Magnification, 480*

repeated for a period of five or six days, with the result that great numbers of intestinal cells are destroyed. Ultimately, the agametes grow into gametocytes, which are of two kinds—one is well stored with food substance and becomes the macrogamete, or egg-like sexual element (Fig. 61 *f* and *g*); the other, after repeated nuclear divisions, forms a great number of delicate, minute, motile microgametes, or spermatozoa-like cells (Fig. 61 *h*, *i*, and *j*). A macrogamete is then fertilized by a microgamete and the resultant zygote immediately secretes a

fertilization membrane which becomes the outer capsule or *sporocyst* of the fertilized cell (Fig. 63). The nucleus, formed by the union of the male and female cells, or the amphinucleus of the



Fig. 62—THE ASEXUAL SPORULATION OF EIMERIA STIEDAE

An intestinal coccidian, parasitic in the rabbit. Each coccidian has divided into a number of spores which appear somewhat like a bunch of bananas. Each spore is able to reproduce the cycle

From a photomicrograph by the author  
Magnification, 700

zygote, now divides twice, and cells form about the four nuclei that result. Each of these four cells—called *sporoblasts*—now secretes its own individual capsule, within which two germs, or sporozoites, are formed by division. The sporocysts, with their sporoblasts and eight sporozoites, are passed out with the feces and sooner or later are eaten by other centipedes, and the history is repeated.

In such a life cycle, auto-infection, or spread of the infection in the same host, is brought about by asexual reproduction of the parasite, while the spread of the infection among different hosts is the result of sexual reproduction. There are thus two cycles, one asexual, the other sexual, and these frequently occur in different types of animal hosts. The asexual cycles of malaria organisms, for example, occur in mammals and birds; the sexual cycle in mosquitoes; the giant gregarine *Porospora* alternates between lobsters and the mussel, *Mytilus edulis*; the coccidian *Aggregata* alternates between crabs and cephalopod molluscs (squid, cuttle-fish, etc.).

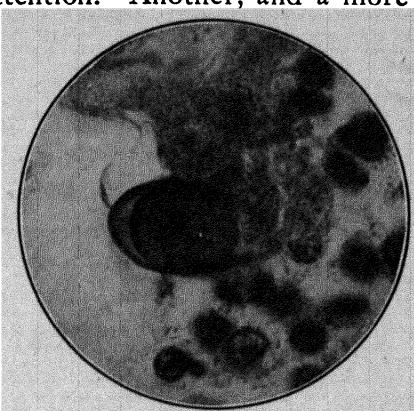
Parasitism is thus an important branch of the science of

protozoology and a biological phenomenon to which many protozoologists give their exclusive attention. Another, and a more

Fig. 63—AN INTESTINAL PARASITE OF THE RABBIT

The fertilized egg of *Eimeria stiedae* in its sporocyst

*From a photomicrograph by the author*  
*Magnification, 650*



fundamental series of biological phenomena upon which light is thrown by the study of protozoa, is outlined in the following chapter.

## CHAPTER XI

### ORGANIZATION, VITALITY, AND MORTALITY

#### LIFE, A COMPLICATED PHENOMENON

A YOUNG organism, recently emerged from a cyst, or just after conjugation, is in perfect condition to respond to the stimuli of its normal environment. The initial changes of the fundamental organization brought about through absorption of water and by oxidation, have already resulted in the formation of the characteristic structures of the derived or adult organization. As soon as these are formed the normal life of the organism begins. Through constant oxidations and other chemical processes, whereby energy for the varied movements and activities of the organism is provided, various substances are broken down and different substances are formed. Through movement food substances are taken in; these are hydrolyzed and distributed throughout the protoplasm in the form of nitrogen-holding proteoses,\* amino acids, and simple carbohydrates like grape sugar. Some of these are utilized in the up-building and growth of the protoplasmic constituents, but much is stored up as granules of albumen and starch-like products, which, together with lipoid (fat-like) substances, become the energy-holding reserves which are the first to disappear under conditions of starvation.

By reason of these continued activities of waste, repair, and growth the protoplasm is constantly changing in composition until an optimum is reached, at which time new activities are set up.

#### PERIODIC REORGANIZATION

These are the activities of division, which are fairly well known morphologically, but about the causes of which we are

\* Proteoses, any of a class of intermediate soluble products formed from proteins by digestion with gastric and pancreatic juices, or by the hydrolytic action of acids and alkalies.

entirely ignorant. Through observation it has been determined that practically each of the essential protoplasmic constituents divides—plastids, chromatin granules, kinetic elements, nucleus, and finally the cell as a whole. Metaplastids, reserve granules, and in general, structures of the derived organization, do not divide, but are either passively distributed to the daughter cells or are absorbed into the protoplasm to be replaced later through the metabolic activities of the young cells.

Cell division is thus a complicated and deeply seated series of phenomena in the life of the organism, and results in a general housecleaning and restoration of the fundamental protoplasmic organization. Endomixis (see page 87) usually accompanied by encystment, has a similar but more far-reaching effect, for the cytoplasm is thereby restored with nucleo-proteins and the nuclear apparatus is renewed. In both cell division and endomixis the result is restoration of vitality through reorganization of protoplasmic materials.

It would seem that this cycle of metabolic activities and periodic reorganization should be adequate to maintain a protozoan through an indefinitely continued existence in a suitable environment. Such indeed was the conclusion expressed by the German biologist, August Weismann, in his essays on life and death written in 1880-1882, in which he argued that protozoa are potentially immortal and that natural death is the penalty which metazoa have to pay for their differentiation into somatic\* and germinal † protoplasm. This idea is apparently borne out by the cultivation by the German biologist, Max Hartmann (1876- ) of the phytoflagellate, *Eudorina elegans*, for a period of fifteen years without fertilization or endomictic processes—division and reorganization alone being adequate for continued vitality. Similar evidence is furnished by the ten-year culture by the German biologist, Karl I. Belar (1895-1931), of the protoplasm of *Actinophrys sol*, a heliozoan, without fertilization and without diminution of vitality. Still further evidence is furnished by the continued life of all animal flagellates where no sexual or fertilization phenomena are known.

\* Somatic protoplasm in metazoa, those cells of the body of an individual which compose the tissues, organs, and parts of that individual; these are the cells which die and cause the death of the individual.

† Germinal protoplasm, the germ cells, which may live and carry on the race.

Although Weismann's conclusion, with some modifications, is undoubtedly correct so far as some types of protozoa are concerned, it is not correct for the majority of protozoa. Protoplasmic differentiation in some types may be carried through continued metabolism to a point where recovery through house-cleaning by division is impossible. Metabolic activities gradually weaken, divisions become more infrequent, and finally the organisms lose the power to divide at all. Such last individuals of a race may struggle along for a few days or even weeks; in one case an individual survived for three months without division, but all finally die a "natural death." Without going into the long history of isolation culture experiments, which were started by Maupas in 1887 in an attempt to disprove Weismann's assertion in regard to "immortality" in protozoa, I will cite one recent experiment which has a direct bearing on the problem.

#### TESTING FOR VITALITY

*Uroleptus mobilis* is a ciliated protozoan of relatively simple structure which lives perfectly well when isolated in a drop of hay infusion and flour (Fig. 50, page 84). A single ex-conjugant was isolated in November, 1917, and its protoplasm was observed and studied daily for a period of more than ten years. At the outset it divided approximately seventeen times in ten days, and the division rate, by ten-day periods, was adopted as a measure of vitality. If we plot the numbers of divisions in successive ten-day periods we obtain a curve of vitality, such as that shown in Figure 64, which is a composite graph of the life histories of twenty-three ex-conjugants. The initial vitality was a little more than fifteen divisions in ten days; after five months it had steadily fallen to ten divisions in ten days, after eight months to three divisions, and the organisms were all dead at the age of thirteen months. This history indicates a slowly changing protoplasmic organization in which the vital activities were carried on with increasing difficulty until they stopped altogether. It is evident that cell division does not effect a complete reorganization in *Uroleptus* and that the daughter cells, after each division, start out with a potential vitality lower than that of the parent cell.

It must be remembered that in such an experiment as here

outlined the individuals are isolated after each division, a treatment which, for some reason which no one has explained, prevents encystment or conjugation and the reorganization which

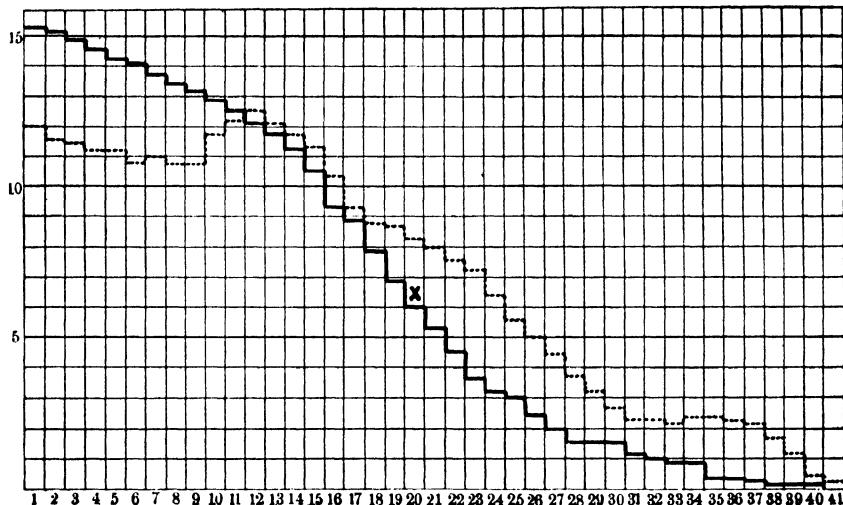


Fig. 64—A VITALITY CURVE OF *UROLEPTUS MOBILIS*

The spaces numbered 1, 2, 3, etc., represent successive 10-day periods; the horizontal rules represent the average number of divisions in ten-day periods. Thus in the first ten-day period the race divided 15.3 times; in the 20th period they divided only six times. The dotted-line curve is the history of a single race, while the solid curve is an average of many races

*From a graph made by the author*

follows those processes. At any time after the first sixty days, if reserve individuals, all of which are closely related to the isolated individuals, are put together in a culture dish, they will conjugate. Reorganization is effected and the ex-conjugant starts a new cycle with the optimum division rate. Thus at the point marked **X** on the graph, when the parent protoplasm was dividing at the rate of six divisions in ten days, one of the ex-conjugants starting a new cycle was dividing at the rate of sixteen divisions in ten days, and under exactly the same environmental conditions. For a period of ten years and by similar procedures one hundred and thirty-five cycles of ex-conjugants were followed through to the death of the cells, each cycle running out in approximately one year. Vitality of that particular bit of protoplasm, isolated in 1917, was maintained at the constant level by periodic reorganizations by conjugation as long as the experiment continued.

**WHEN DOES A YOUNG PROTOZOAN BECOME AN ADULT?**

Conjugation in any series is not possible until there has been approximately two months of continued metabolic activity. This indicates that protoplasmic differentiation must have progressed to a stage corresponding to what we call sexual maturity in metazoa, when fusion of germ cells becomes possible. In *Uroleptus mobilis* there is no structural or visible indication of this differentiation, but in Foraminifera, in Radiolaria, in nearly all Sporozoa, and in many plant flagellates, a similar stage of sexual maturity in the life cycle is indicated by definite division phenomena which differ from the customary method of division and differ also from the meiotic phenomena but which occur only at this period. In Foraminifera, Radiolaria, and Gregarines, minute cells termed gametes, are formed from the nuclei and portions of the cytoplasm of the parent organism (Fig. 44, page 77). Such gametes fuse two by two, and the zygote thus formed gives rise to one or more vegetative individuals with optimum vitality. There is considerable evidence to indicate that nuclear fusion in ciliates is a reminiscence of such an ancestral gamete brood.

**DIFFERENTIATION INTO MALE AND FEMALE**

In ciliates, with the exception of the Vorticellidae, there is nothing in the structure of the conjugants which enables us to speak of one as male and of the other as female. The same is true in all protozoa where isogametes (similar gametes) are formed. Yet the fact that two apparently identical cells approach and fuse may be interpreted as an indication that some difference, which may be called sexual, exists between them. In other cases, particularly in the Volvox group among plant flagellates and in Coccidia among the Sporozoa, protoplasmic differentiation resulting from continued metabolism follows two definite paths, one leading to large forms with sluggish, or no movement, and abundant reserves of nutriment; the other leading to a gametocyte in which food reserves are slight and which gives rise to a multitude of minute, active microgametes. Thus in protoplasmic differentiation the larger form, or macrogamete, is equivalent to an egg cell and the minute, active forms to

spermatozoa. In some rare cases, as for example in *Cyclospora karyolytica* and in some species of *Volvox*, in which there are male and female colonies, this differentiation is thrown back into the fundamental organization, possibly centered in the nucleus, and individuals develop from the outset as male or female.

Such extreme differentiation leading to the formation of distinct male and female gametes means an upset of the balance in the life activities of the organism and the gametes die unless the balance is restored by fusion. In ciliates, such as *Uroleptus mobilis*, differentiation of the two sexes is not far enough advanced at periods of sexual maturity to deter metabolic activities, but in types that go beyond this stage cumulative differentiation of the sexual reproductive cells leads to an increasing inhibition of their vital activities and ultimately to death unless reorganization supervenes, a reorganization that can only be brought about by sexual union of the male and female gametes.

#### CONTINUITY OF LIFE

This power of restoration to the condition of the fundamental organization that underlies reproductive processes is a basic characteristic of living substance and is responsible, apparently, for the continuity of life through past ages. The cyclical phenomena described above furnish a hint as to the factors controlling such longevity of protoplasm. A naked *Amoeba proteus* or a *Uroleptus mobilis* represents protoplasm which has been continuously living since the beginnings of life and protoplasm which, barring accidents, has the possibility of living indefinitely in the future. Obviously the same statement is true of all other living things. With Protozoa, however, the concept is more spectacular because of the relative simplicity of their derived organizations. Longevity or continued life of this protoplasm is dependent upon the periodic clearing away of the products of protoplasmic activity both in the form of derived structures and of metabolic products. This restoration to the original condition of the fundamental organization is accomplished by the destructive phenomena ac-

companying cell division, endomixis and conjugation, all of which are characteristic of Protozoa but not of Metazoa. This, as I see it, is a fundamental difference between Protozoa and Metazoa. In Metazoa the germ cells alone possess the fundamental organization with its potential of immortality. These, after fusion, give rise by growth and differentiation to other germ cells and to the vast edifice of somatic cells, tissues, and organs of the derived organization. These are not removed at intervals and replaced by new structures as in Protozoa, but when their potential of vitality is exhausted they die a natural death and the contained germ cells, deprived of protection and nourishment, are killed and disappear with them. Other germ cells meanwhile have left the body, been fertilized and develop as sons and daughters to continue the longevity of the fundamental organization. Since other methods of reorganization are possible among the smallest living things, fertilization for the most part is not essential for continued vitality. Amphimixis (sexual union), however, introduces new possibilities into fundamental organizations. This provides an opportunity for mutations and for the establishment of derived organizations which may be useful (or harmful) in the universal struggle for existence.\*

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\* For the further development of the theme contained in this paragraph see: G. N. Calkins. The Factors controlling Longevity in Protozoan Protoplasm. In Biological Bulletin Vol. LXVII, Dec., 1934.

## SUGGESTIONS FOR FURTHER READING

Prepared by the Author

### TEXTBOOKS

<b>BIOLOGY OF THE PROTOZOA</b> — <i>Gary N. Calkins</i>	<b>LEA</b>
A book for more advanced students; illustrating by protozoa general and fundamental biological principles.	
<b>BRITISH FRESH-WATER ALGAE</b> — <i>G. S. West</i> ; revised by <i>F. E. Fritsch</i>	<b>MACMILLAN</b>
A concise account of the structure, habits, and life-histories of the algae found in the fresh waters of the British Isles.	
<b>BRITISH DESMIDIACEAE</b> — <i>W. West</i> and <i>G. S. West</i>	<b>ROYAL</b>
An excellent treatment, well illustrated, of the desmids.	
<b>GENERAL BACTERIOLOGY</b> — <i>E. O. Jordan</i>	<b>SAUNDERS</b>
A readable, not too technical, general account of bacteria.	
<b>HANDBOOK OF PROTOZOLOGY</b> — <i>R. R. Kudo</i>	<b>THOMAS</b>
A good, illustrated account of the more common forms of protozoa.	
<b>SYNOPSIS OF BRITISH DIATOMACEAE</b> — <i>W. Smith</i>	<b>VAN VOORST</b>
A good guide to the common forms of the diatoms, particularly those of fresh water.	

### NON-TECHNICAL BOOKS

<b>BOOK OF THE MICROSCOPE</b> — <i>Gerald Blavins</i>	<b>LIPPINCOTT</b>
A good book to interest beginners in the wonder-revealing powers of the microscope.	
<b>FRESH-WATER BIOLOGY</b> — <i>H. B. Ward</i> and <i>G. C. Whipple</i>	<b>WILEY</b>
Illustrated descriptions of fresh-water microscopical life; very convenient and useful.	
<b>HUNTING UNDER THE MICROSCOPE</b> — <i>Arthur E. Shipley</i>	<b>MACMILLAN</b>
Microscopical animal life in fresh-water ponds and at the seashore.	
<b>PLANT LIFE</b> — <i>Charles A. Hall</i>	<b>MACMILLAN</b>
A clear account of plant life from the simplest microscopic forms to specialized flowering plants.	
<b>LIFE</b> — <i>Arthur E. Shipley</i>	<b>MACMILLAN</b>
In clear, simple terms the author explains the facts of protoplasm, cells, food, digestion, respiration, etc., of plants and animals.	

### REFERENCE BOOKS

<b>THE BEHAVIOUR OF LOWER ORGANISMS</b> — <i>H. S. Jennings</i>	<b>COLUMBIA</b>
A readable account of the nature of stimuli and the reactions of microscopic animals.	
<b>BRITISH FRESH-WATER RHIZOPODA</b> — <i>James Cash</i> and <i>G. H. Wailes</i>	<b>RAY SOCIETY</b>
A good description of the common species of pseudopodia-bearing protozoa found in the British Isles.	
<b>THE MICROSCOPY OF DRINKING WATER</b> — <i>George C. Whipple</i>	<b>WILEY</b>
A good account of the microscopic organisms causing odors and tastes in drinking waters.	

### PHILOSOPHICAL BOOKS

<b>LIFE AND DEATH, HEREDITY AND EVOLUTION IN UNICELLULAR ORGANISMS</b>	<b>BADGER</b>
<i>H. S. Jennings</i>	
A discussion of these fundamental concepts as illustrated by the Protozoa.	
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A good account of those fundamental concepts which play a large part in the philosophy of biology.	

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## GLOSSARY

[Only those terms are defined in this glossary which either are not explained in the text, or are explained once and used again several pages beyond the explanation.]

**AGAMETES**: asexual reproductive cells which develop, without fertilization, into an adult organism.

**ALGAE**: the most primitive green plants; they live in both fresh and salt water or in moist locations.

**AMINO ACID**: an acid in which a portion of the nonacid hydrogen has been replaced by a nitrogen-hydrogen combination. Many of the amino acids are important products when proteids decompose.

**AMPHIMIXIS** (from two Greek words, *amphi*, meaning "of both kinds," and *mixis*, meaning "a mingling") : the union of the germ-plasms of two individuals in sexual reproduction.

**AMPHIMONAD** (from two Greek words, *amphi*, meaning of both kinds," and *monad*, meaning "alone," or "unit") : a technical term used in protozoology to designate a simple animal-flagellate with two equal flagella.

**AMPHINUCLUS**: a new nucleus formed by the fusion of two nuclei, each of which had but half the normal number of chromosomes.

**ANTIBODY**: any of various bodies or substances in the blood which act in antagonism to harmful foreign bodies, such as toxins.

**ANTITOXIN**: any of certain complex soluble chemical compounds occurring in the blood, either normally or under certain special conditions, and having the power of neutralizing some specific poison—especially a compound capable of neutralizing one of the poisons produced in the body by pathogenic bacteria, and hence conferring immunity, or conducing towards recovery, from the disease caused by the bacteria.

**ANUS (adjective, ANAL)**: the posterior opening of the alimentary canal, or in protozoa, a permanent pore in the cell through which indigestible remains are voided.

**ARBOROID**: in the form of a tree or bush; used in protistology to indicate a branching type of colony.

**ATROPHY** (from the Greek, meaning "not to nourish") : a stoppage of development of a part or organ (often followed by loss in size or even complete disappearance) incidental to the normal development or life of an animal or plant.

**BASAL BODY**: a granule in the cell from the substance of which a flagellum or cilium grows.

**CARBOHYDRATE**: any of a group of neutral compounds, including the sugars, starches, celluloses, etc., composed of carbon, hydrogen, and oxygen, and characterized by containing six, or a multiple of six, carbon atoms, combined with hydrogen and oxygen in the proper proportion to form water.

**CATALYSIS (plural CATALYSES)**: the speeding up of a reaction which has been induced by the presence of a substance, called the catalytic agent or catalyst, which itself appears to remain unchanged. Originally the term was applied to decompositions only.

**CATALYST**: a substance which causes the break-down of other substances.

**CATENOID**: like a chain; linked together in such a way as to give the appearance of a chain.

**CAUDAL**: like or pertaining to a tail.

**CELLULOSE**: an inert substance constituting the chief part of the solid framework of plants, of ordinary wood, linen, paper, etc. It is also found to a slight extent in certain animals. It is a carbohydrate of the same percentage composition as starch, and is convertible into starches and sugars by the action of heat and acids. When pure it is a white mass without crystals.

**CHLOROPHYLL:** the green coloring matter in plants.

**CHLOROPLASTID:** a plastid forming and containing chlorophyll.

**CHROMATIN:** a protoplasmic substance found in the nucleus of cells; regarded by many as the physical basis of heredity: It exists in small granules, which at cell division become aggregated into chromosomes.

**CHROMATOID GRANULES:** granules like those which contain chromatin.

**CHROMATOPHORES:** the color bodies found commonly in plant cells; they include both green chloroplastids and red or yellow chromatoplastids, and vary greatly in form and size. In certain lower forms of algae they afford sustenance to the organism.

**CHROMOSOME:** one of the small bodies into which the chromatin of a cell nucleus resolves itself previous to cellular or nuclear division.

**CILIA** (*singular, CILIUM*): hairlike processes, found on many cells, capable of a vibratory or lashing movement. Unlike pseudopodia (*q.v.*) cilia are permanent processes, and in many cases keep in incessant motion. In free-swimming unicellular organisms, and some small multicellular forms, cilia serve as organs of locomotion. In the higher animals their usual function is to produce a current of fluid. For example, in man ciliated cells line the nasal cavity, the trachea, the bronchi, etc., and by a constant propulsion toward the nose assist the removal of mucus and dust particles.

**CILIATES:** a sub-phylum of protozoa having cilia in the immature stages (*Suctoria*) or throughout life (*Ciliata*).

**CIRRUS** (*plural, CIRRI*): in protozoology coalesced cilia forming complex motile organs which are circular in cross section.

**COCCOID BODIES:** reproductive bodies occurring in spirochaetes; equivalent to bacteria spores.

**COLUMELLA:** in zoology and anatomy, any of various parts which can be likened to a column (*cf. newel post in a winding staircase*).

**CONJUGATION:** the temporary fusion of two unicellular organisms, involving a union of their nuclei or an interchange of nuclear material. Though male and female cells are not distinguished, the process is analogous to fertilization among higher organisms.

**CORTEX:** the outer or superficial part of an organ, especially the outer layer of gray matter of the brain; also the outer part (ectoplasm) of certain organisms, as some of the protozoa, the internal portion being called the endoplasm.

**CORTICAL ZONE:** the outer layer of living protoplasm; *see* CORTEX.

**CYTOLogy:** the branch of biology which treats of cells.

**CYTOPLASM:** the protoplasm of the cell exclusive of the nucleus.

**DECOMPOSE:** to resolve into original elements or into simpler compounds.

**DERIVED ORGANIZATION:** the final stage of development of an organism.

**DESMID** (from the Greek word meaning "chain"): an unicellular alga.

**DIATOM** (from the Greek, meaning "cut in two"): any unicellular alga belonging to the order Bacillariales; diatoms are remarkable for a silicified cell-wall which persists as a skeleton after the death of the organism and forms kieselguhr. Diatoms are always found on submerged objects (wood, stones, etc.) to which they may impart a slimy feeling. Kieselguhr is a fine, usually white powder, and is used as an absorbent in dynamite and as a polishing material.

**DORSAL:** pertaining to, or situated near or on, the back (dorsum) of an animal or of one of its parts—opposed to *ventral*.

**ELECTROLYTE:** a compound substance which can be decomposed (separated) into its elements by an electric current. Salts, as a rule, disassociate readily, acids in various degrees. Substances which do not conduct an electric current are called nonelectrolytes.

**ENCYSTMENT:** the process of forming a cyst or of becoming enclosed in a capsule; the state of being encysted.

**ENDOMIXIS** (*adjective, ENDOMICRIC*; from the Greek *endo*, meaning "within" and *mixis*, a mixing): dissolution of the macronucleus (*q.v.*) of an infusorian and its reorganization from micronucleus (*q.v.*) without the intervention of conjugation (*q.v.*).

**ENDOSMOSIS:** *see* OSMOSIS.

**ENZYME:** any of a number of complete organic substances capable of effecting catalysis (*q.v.*); enzymes are sometimes called unorganized, unformed, or

chemical ferments in distinction to so-called organized or living ferments, such as yeasts.

**EPITHELIUM** (*plural, EPITHELIUMS; adjective, EPITHELIAL*; from the Greek *epi*, meaning "on" or "upon," and *phelē*, meaning "nipple") ; a cellular tissue covering a free surface or lining a tube or cavity, and consisting of one or more layers of cells with scarcely any intercellular substance, so that the cells form a practically unbroken sheet or membrane.

**EX-CONJUGANT**: either one of the two organisms which have recently been temporarily united in conjugation (*q.v.*).

**EXCRETION**: the process of ridding the body of products of destructive metabolism (*q.v.*). Excretion, technically, must be distinguished from defecation, the process of ridding the body of indigestible remains.

**EXOSMOSIS**: *see OSMOSIS.*

**EYE-SPOT**: a small pigment body in certain unicellular algae, supposed to be sensitive to light.

**FERMENT**: an agent capable of producing fermentation; *see also ENZYME.*

**FLAGELLATE**: a one-celled organism, so named because one, two, or more flagella are attached to the cell and serve as swimming organs. *Flagellum* is the Latin for "whip."

**FLAGELLUM** (*plural, FLAGELLA*): *see FLAGELLATE.*

**FRUSTULE**: the siliceous shell of a diatom, composed of two valves, one overlapping the other.

**GAMETE**: a sexual cell—either egg or sperm.

**GAMETOCYTE** (from the Greek, *gamen*, meaning "to marry" and *kytos*, meaning "a hollow vessel") : technically the term is used to designate the cell which gives rise to the gametes (*q.v.*).

**GENE**: a hypothetical unit in a chromosome (*q.v.*) of the cell which has a specific influence on a certain adult characteristic; the unit of hereditary transmission.

**GENETICS**: the science which deals with the principles of heredity and variation and the processes involved in the origin of species, races, and individuals.

**GERMINATION**: (1) beginning of growth or development of a spore; (2) resumption of growth by the embryo of a plant.

**GERM-PLASM**: the substance composing the germ cells by which hereditary characters are transmitted.

**GRAPE SUGAR**: dextro-glucose; called grape sugar because found in ripe grapes; it occurs in very many plants and in animal organisms; it is about half the sweetness of cane sugar. Its formula is  $C_6H_{12}O_6$ .

**HERMAPHRODITE**: an individual having both male and female reproductive organs.

**HISTOLOGY**: the branch of science which treats of the minute structure of normal animals and vegetable tissues as discernible with the aid of the microscope; microscopic anatomy.

**HOLOPHYTIC** (from two Greek words meaning *all* and *plant*): wholly or distinctively vegetable in nutrition; obtaining food after the manner of a green plant; the process involves photosynthesis (*q.v.*).

**HOLozoic** (from two Greek words meaning *all* and *animal*): wholly or distinctively like an animal as to nutrition; the process involves the digestion of solid food substances.

**HYDROLYSIS**: a chemical process of decomposition (*see DECOMPOSE above*) involving the addition of the elements of water. Thus crystalline sugar when dissolved has been hydrolyzed.

**INCLUSION**: a foreign body, usually of minute size, inclosed in a larger mass; technically a foreign body inclosed in a cell.

**INFUSION**: water containing substances extracted from plant or animal matter. Ordinary tea is an infusion.

**INOCULATION**: the introduction of bacteria or immune serum into living organisms; especially the introduction of disease germs or their products into a person in health, by inserting them in his blood, in order to induce a mild form of the disease which will secure immunity from future attacks, or will combat the disease already present.

**IRRITABILITY**: the response of an organism to stimuli; it may be visible to the eye in the form of movement, or it may be molecular and hence invisible.

**KINETIC ELEMENTS**: special bodies and fibers in a cell which have to do with movement and with the transmission of stimuli to motile organs.

**LEUCOSIN**: a chemical substance of unknown origin composing certain glistening bodies in the cells of some of the plant-flagellates.

**MACROGAMETE** (or **MEGAGAMETE**): the larger, or female, of two gametes.

**MACRONUCLEUS** (*plural*, **MACRONUCLEI**): the larger of the two kinds of nuclei of infusoria.

**MATRIX** (from the Latin, *mater*, meaning "mother"): substance from which structures are formed, or in which they are embedded.

**MATURATION**: the final series of stages in the formation of the egg or the sperm by which its nucleus is prepared for union.

**MEIOSIS** (*adjective*, **MEIOTIC**), the process of being made smaller; technically the processes of nuclear division in maturation (*q.v.*) of the germ cell.

**METABOLISM**: the chemical changes proceeding continually in living cells, by which the energy is provided (destructive metabolism) for the vital processes and activities (*see page 88*), and new material is prepared and assimilated to repair the waste (constructive metabolism).

**METAPHYTE** (*plural*, **METAPHYTES**): a multicellular plant.

**METAPLASTID**: a product of cell activity which remains stored up in the protoplasm.

**METAZOA** (*singular*, **METAZOAN**): multicellular animals.

**MICROGAMETE**: the smaller, or male, of two gametes.

**MICRONUCLEUS** (*plural*, **MICRONUCLEI**): the smaller of the two kinds of nuclei of infusoria.

**MIXOTROPHIC**: deriving nourishment from a combination of any two or even all (three) of the following methods of food-getting: holophytic, holozoic, and saprozoic.

**MONAD** (from the Greek word meaning "alone" or "unit"): a minute, simple flagellated organism.

**MORPHOLOGY** (*adjective*, **MORPHOLOGICAL**): the branch of biology dealing with the form and structure of animals and plants; the study of the forms, relations, changes, phylogenetic (*see PHYLOGENY, below*) development of organs apart from their functions.

**MOTILE**: exhibiting, or capable of, spontaneous movement.

**MOTILE PHASE**: the stage of the life history of an organism during which it is free swimming.

**MUTATION**: a sudden heritable change in some aspect of the organism, due to an alteration in the hereditary material.

**MYONEMES**: thread-like muscular elements found in gregarines and in ciliated protozoa.

**NUCLEOPROTEINS**: any of a class of compound proteins (*q.v.*) found in nearly all cell nuclei, in protoplasm, etc.

**NUCLEUS** (*plural*, **NUCLEI**): from the Latin meaning "little nut" or "seed"): the central body, with specialized structures and functions, found in nearly all cells.

**NUTRITION**: the aggregate of activities having to do with obtaining food, digesting and assimilating it, and adding it to the protoplasmic substances already present.

**OBLIGATORY**: in biology, the state of being limited to a single life condition, *e.g.*, parasites; opposed to "facultative," which means having the power to live under different conditions.

**OSMOSIS**: the passing of a fluid through a porous partition, such as a membrane, to mix with another fluid on the other side. The inward flow is called **ENDOSMOSIS**, and the outward flow **EXOSMOSIS**.

**OPTIMUM** (from the Latin, meaning "best"); the most favorable condition as to temperature, light, moisture, food, etc., for the growth and reproduction of an organism.

**OXIDATION**: the process of combining with oxygen, or with more oxygen.

**PALMELLA PHASE**: a quiescent phase in the life history of a plant flagellate during which the organism is embedded in a gelatinous substance.

**PARAMYLUM**: a carbohydrate ( $C_6H_{10}O_5$ ), allied to starch, found in unicellular organisms.

**PARTHENOGONIDIUM** (*plural*, **PARTHENOGONIDIA**): (1) an individual which can be produced by a colony by one of the asexual methods—cell-division, spore-formation, budding, etc., but does not include the self-fertilization of hermaphroditic organisms; (2) an asexually produced individual.

**PATHOGENIC** (from the Greek word *pathos*, meaning "suffering," and the suffix *genic*, meaning "generating" or "producing") : causing disease.

**PELICLE** (from the Latin, *pellicula*, the diminutive of *pellis*, meaning "skin") : a thin lifeless membrane about a free-living cell.

**PERIPHERY** (from the Greek, *peri*, meaning "around," and *phierein*, meaning "to bear" or "to carry") : the external boundary or superficial parts of any body.

**PERIPLAST**: a lifeless cell membrane, equivalent to "pellicle" (q.v.).

**PERIPLASM** (*adjective*, **PERIPLASTIC**) : enclosing or outer protoplasm.

**PHOTOSYNTHESIS**: the process by which carbohydrates are formed from inorganic compounds by the chlorophyll of plants in sunlight.

**PHYLOGENY**: the race history of an animal or a plant type, in distinction from the development of the individual organism (*ontogeny*).

**PHYLUM** (*plural*, **PHYLA**) : one of the primary divisions of the animal or vegetable kingdom, so called because the members are assumed to have a common descent.

**PLASTID**: (1) a unit of protoplasmic matter: (2) any of certain small bodies of specialized protoplasm lying in the cytoplasm (q.v.) of some cells, especially plant cells and those of certain protozoa, and serving in many cases as organs or places of special activities concerned with metabolism (q.v.).

**PROPHYLAXIS**: the art of guarding against, preserving from, or preventing disease; observance of the rules necessary to preserve health.

**PROTEINS** (from the Greek word, *proteuin*, meaning "to be first") : any of a class of extremely complex substances which are of first importance in physiological chemistry, being essential constituents of all living cells, both animal and plant, and also of the diet of the animal organism.

**PROTEOLYSIS** (*adjective*, **PROTEOLYTIC**) : the hydrolysis (q.v.) of proteins with the formation of simpler and soluble products, as occurs in digestion.

**PROTISTA**: the unicellular organisms collectively, including both the one-celled animals and the one-celled plants.

**PROTOPLASM**: the living substance of which animals and plants are essentially composed.

**PROTOPHYTE** (*plural*, **PROTOPHYTA**; *adjective*, **PROTOPHYTIC**) : an unicellular plant.

**PROTOZOAN** (*plural*, **PROTOZOA**; *adjective*, **PROTOZOAN**) : a minute animal, generally consisting of a single cell, or of a colony, all the cells of which are fundamentally alike.

**PSEUDOPODIUM** (*plural*, **PSEUDOPODIA**) : a temporary protrusion or retractile process of the protoplasm of a cell, especially of an unicellular organism. Pseudopodia may have a fairly definite filamentous form, sometimes fusing with others to form a network, or they may be irregularly lobate as in the ameba. They serve various purposes, especially as means of locomotion or for taking up food.

**REPRODUCTION**: the means by which a life-form is continued and multiplied.

**REPRODUCTION BY DIVISION**: the simplest, or fundamental, form of multiplication. In the last analysis all reproduction is by division.

**SAPROPHYTE** (*adjective*, **SAPROPHYTIC**) : a plant living on dead and decaying plants or animals.

**SAPROZOIC**: living on proteins and carbohydrates in solution.

**SOMATELLA**: usually a transient stage in development in which the nucleus has divided several times, while the cell body remains undivided.

**SPOROBLAST**: in certain protozoa (the sporozoa), a cell which divides into sporozoites (q.v.).

**SPOROZOITE**: in sporozoa, a small active, usually elongate, spore, especially one of those produced by division of the sporoblasts (q.v.) into which the zygote divides.

**SPORULATION**: the division into many small spores, especially after encystment.

**STAINING**: a complicated process for the purpose of coloring certain parts of the cell (e.g., the nucleus).

**SYMBIOSIS** (*adjective*, **SYMBIOTIC**) : the living together in more or less intimate association or even close union of two dissimilar organisms. Ordinarily the term is used in cases where the association is advantageous (often necessary) to one or both, and not harmful to either.

**SWARMER**: a spore with the ability to move independently, usually by means of flagella.

**SYNTHEZIZING**: the putting of two or more elements or simple compounds together to make a new compound; the opposite of analyzing.

**THALLOPHYTES**: plants of very diverse habits and structures, including the algae, the fungi, and the lichens.

**TOXIN**: any of a class of poisonous substances formed as secretion products of vegetable and animal organisms. They are distinguished from inorganic poisons by their property of inducing the formation of an anti-toxin when introduced into a suitable animal and, in most cases, by an incubation period before symptoms of poisoning appear.

**TYPICAL**: combining or exhibiting the essential characteristics of a group.

**VACCINATION**: the inoculation with a weakened virus as a preventive measure against disease.

**VACUOLE**: a small cavity containing a watery fluid; it is characteristic of plant cells and protozoa, but occurs also in cells of higher animals. In plants vacuoles contain the cell sap; in protozoa they may contain secretions of the protoplasm, or substances about to be excreted, or food in various stages of digestion and assimilation.

**VACUOLE, CONTRACTILE**: a vacuole found in the protoplasm of many unicellular organisms, which gradually increases in size, and then suddenly collapses, often making regular pulsations. Its function is believed to be respiratory or excretory.

**VACUOLIZATION**: the state of containing one or more vacuoles.

**VENTRAL**: designating, pertaining to, or situated on or toward that surface of the body which in man is anterior, but which in most vertebrates and invertebrates is the lower surface—opposed to *dorsal*.

**VIRUS**: the poison (*contagium*) of an infectious disease.

**ZOO $\ddot{\text{o}}$ SPORES**: reproductive bodies with the ability to move, usually by means of flagella; used by botanists for filamentous algae.

**ZYGOSPORE**: a spore formed by the conjugation of two similar gametes; a term used by botanists in the same sense as the term zygote (*q.v.*) is used by zoologists.

**ZYGOT, or ZYGOTE**: the product of the union of two sexual cells; a fertilized cell.

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## KEY TO PRONUNCIATION

ā as in dāy	ē as in mēte	ī as in tīme	ō as in nōt
ā " " senātē	ē " " ēvent	ī " " īdea	ō " " lōrd
ă " " ādd	ĕ " " ēnd	ĭ " " ill	
ă " " cāre	ē " " tērm	ī " " fīrm	ū " " ūse
ă " " fār	g = j (gentile)	ō " " öld	ú " " ūnīte
ă " " fāst	g as in get	ō " " öbey	ū " " ūs
			ū " " tūrn

















